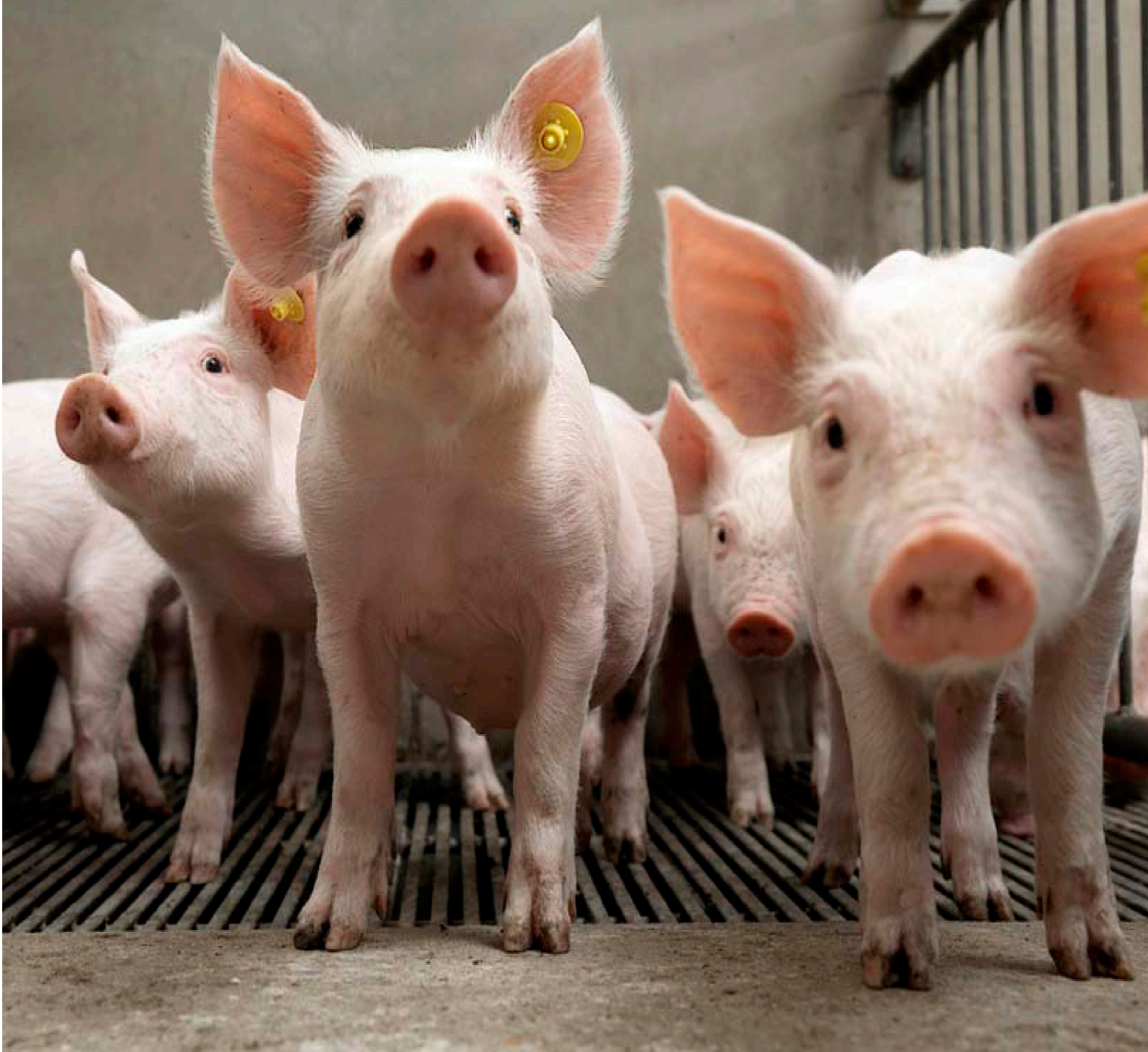

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PUBLIC
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WAGENINGEN
UNIVERSITY & RESEARCH

Impact of calcium content and calcium to phosphorus ratio in diets for weaned pigs

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1 Wageningen Livestock Research
2 ForFarmers
3 De Heus

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Samenvatting
Dit onderzoek is uitgevoerd om de invloed van het calciumgehalte en de calcium/fosfor verhouding in het voer op de groeiprestaties en de mineralen excretie bij biggen te bepalen. Twee experimenten zijn uitgevoerd gedurende een vier of vijf weeks periode waarin speenvoer en biggenopfokvoer werd vertrekt met en zonder microbieel fytase en verschillende Ca en P gehalten. De resultaten zijn gebruikt ter bespreking van de Ca en P normen voor biggen en de consequenties van een verlaging van het Ca-gehalte.

Summary
This study was conducted to determine the influence of calcium (Ca) content and calcium to phosphorous (P) ratio on growth performance and mineral excretion in weaned pigs. Two experiments were conducted during a four or five week period using prestarter and starter diets with or without microbial phytase and varying in Ca and P content. The results are used to discuss Ca and P recommendations and consequences of a reduction in dietary Ca content.

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Public Wageningen Livestock Research Report 1309

Table of contents

Foreword

The research "Impact of calcium content and calcium to phosphorus ratio in diets for weaned pigs" was conducted by Wageningen Livestock Research and private partners De Heus and ForFarmers as part of the Public Private Partnership "Feed4Foodure", and was funded by Vereniging Diervoederonderzoek Nederland (VDN) and the Ministry of Agriculture, Nature and Food Quality (LNV). The authors thank VDN and LNV for their support, and the members of the Cluster "Swine" of VDN for their valuable contribution to the research. The skilful and devoted contribution of staff of the two facilities at which the research was conducted, and of colleagues involved at De Heus and ForFarmers is highly appreciated!

Paul Bikker

Summary

This study was conducted to determine the influence of dietary calcium (Ca) and phosphorous (P) inclusion level and Ca to P ratio (Ca/P) on growth performance and faecal consistency in weaned pigs, Ca and P digestibility and urinary excretion. In addition, the impact on skin damage due to (negative) behavioural interactions between the pigs was registered. The experiments were performed in research facilities of De Heus and ForFarmers with 4 treatments and 16 pens (experimental units) per treatment group and in total 704 (ForFarmers) and 448 (De Heus) newly weaned entire male and female pigs. Each experiment comprised four dietary treatments with (ForFarmers) or without (De Heus) inclusion of 2000 FTU microbial phytase per kg.

1) Control diets, Ca and P at the level of CVB-recommendations
2) Low Ca diets, Ca content based on practical use, P content at the level of control diets (g/kg)
3) Low Ca diets, Ca content based on practical use, low P content with Ca/P as in control diets.
4) High Ca diets, Ca content equal to control diets, high P with Ca/P ratio equal to treatment 2.

The experiments were conducted during a four (ForFarmers) or five (De Heus) week period from weaning. The pigs had free access to a phase 1 (prestarter) nursery diet during two weeks and a phase 2 (starter) diet thereafter. All diets were prepared at one production location from a basal prestarter and starter diet in which endogenous phytase was minimised via heating. Dietary treatments were realised by inclusion of the required amount of limestone and monocalcium phosphate. Titanium dioxide was included as a marker to determine total tract nutrient digestibility of the starter diet by grab sampling during three successive days in the fourth week of the experiment. On the same days, urinary samples were collected to determine Ca and P concentration. The pigs were weighed on day 0, 14, 28 and 35 (De Heus only) of the experimental period. Growth performance was calculated in the prestarter, starter and total experimental period. On days 14, 28 (ForFarmers) and 35 (De Heus) visual damage of skin, tail and ears was registered when weighing the pigs. Faecal consistency was registered using a scoring system from 0 (firm faeces) to 3 (watery diarrhoea) during the first three weeks of the experiment.

In the combined results of the two experiments the lower dietary Ca content (with constant Ca/P) enhanced the ADFI in the 28 d period and the ADG in the first 14 d. Only at location De Heus using phytase-free diets the reduction in dietary Ca enhanced the ADG in the total five-week period. The dietary treatments did not substantially effect faecal consistency. Apparent total tract digestibility (ATTD) of Ca and P was substantially higher at location ForFarmers using phytase-supplemented diets. The reduction in dietary Ca content enhanced ATTD of Ca and only in phytase-supplemented diets also the ATTD of P. The reduction in Ca/P ratio enhanced the ATTD of P at both locations, with more effect in the phytase-free diets. The simultaneous reduction in Ca content and Ca/P (practical diets versus CVB recommendations) enhanced the ATTD of both Ca and P. The realised digestible P contents were lower than calculated in phytase-free diets and close to calculated values in phytase-supplemented diets. The ATTD of Ca was lower than calculated at the high Ca content and close to calculated in diets with low Ca content. The urinary Ca content was drastically reduced and the P content enhanced by a low dietary Ca/P ratio. Phytase affected the magnitude of these effects.

Using CVB recommendations as a reference, the results of this study indicate that dietary Ca content can be reduced without loss in performance. In phytase-free diets this may improve growth performance. The simultaneous reduction in Ca content and Ca/P ratio enhanced the ATTD of P in both phytase-free and phytase-supplemented diets. However, the reduction in Ca content and Ca/P enhanced urinary P content. Subsequent reduction in dietary P content resulted in a (large) reduction in P excretion, and a drastic increase in Ca excretion, indicating a reduction in Ca and P retention and bone mineralisation in the body. The results of our study and published literature in weaned pigs indicate that in diets with adequate P a negative effect of high dietary Ca on growth performance is negligible below STTD Ca/P of 1.4 (approximately 2.3 Ca/STTD P) and small between 1.4 and 1.55 (approximately 2.3 and 2.6 Ca/STTD P). The latter is especially the case in phytase-supplemented diets because of the lower total (gross) Ca content. We recommend to use a STTD Ca/P of 1.4 as a minimum in diets for weaned pigs. A further reduction would likely result in an increase in P losses via the urine and a reduction in bone mineralisation.

1 Introduction

1.1 Background

Calcium (Ca) and phosphorous (P) are essential nutrients for pigs. These minerals are required as major components of bone to provide structure and protection to the body, for a number of physiological processes, e.g. for the nervous system, energy metabolism (ATP) and cell function and multiplication (phospholipids, DNA). To meet the requirements of processes in the body for performance and health and for optimal nutrient utilisation, Ca and P need to be supplied in the required ratio, in particular because of the constant ratio of Ca and P in bone as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). A low ratio between absorbed Ca and P would limit the retention of P in bone and enhance P excretion in the urine. A high dietary Ca to P ratio may reduce the digestion and absorption of P in the digestive tract because of a negative impact on phytase efficacy, P solubility and P absorption (Hu et al., 2021). Based on the retention of Ca and P in the body, endogenous losses and the adopted efficiency of utilisation, Bikker and Blok (2017) derived Ca and P requirements for different categories of pigs. For weaned pigs, a requirement of 3.8 and 3.2 g standardised total tract digestible (STTD) P per EW (unit of energy, 8.8 MJ NE) was derived for week 1-2 and week 3-6 in the nursery period. Based on a digestible Ca to P ratio of 1.55, the required STTD Ca is 5.9 and 5.0 g/EW. Assuming a mean STTD of Ca of 60%, without inclusion of phytase, the total calculated Ca requirement is 9.8 and 8.4 g/EW. The recommendations for Ca and P based on their use for bone mineralisation and retention in the body may exceed the requirements for maximum growth performance. This was illustrated in a dose response study in growing-finishing pigs in which maximum gain was realised at a P inclusion close to CVB recommendations, whereas Ca and P retention in the body continued to increase until the highest dietary P content of 130% of CVB recommendations (Bikker et al., 2013).
For the production of practical diets for nursery pigs, the CVB-recommendations for Ca (Bikker and Blok, 2017) are regarded as high. In practice, it is often preferred to reduce the Ca content in diets for weaned pigs because of the risk of a reduction in feed intake, daily gain and possibly gut health due to the high buffering capacity in high calcium diets. The actual Ca concentration in practical diets may be substantially below the CVB-recommendations for weaned pigs. Indeed in a previous study (Hu et al., 2021) we observed a negative impact of an increasing dietary Ca content from 2.0 to 9.6 g/kg on growth performance (ADG, FCR) of young growing pigs (30-50 kg). This effect was reduced with inclusion of phytase in the diet, suggesting that P-deficiency was a major cause of the negative effect of a high Ca content. Thus, availability of P and the presence of microbial phytase seems to mediate the potential negative effect of Ca. Nonetheless, the reduction of dietary Ca content substantially below recommendations as observed in practical diets for weaned pigs may imply that the Ca/P is too low for optimal P-utilisation and bone strength and may enhance P excretion in the urine. The suboptimal Ca/P in practical diets could then be improved by increasing the Ca content to increase the bone strength or reducing P content for optimal P utilisation.
This study was conducted to clarify the aspects of Ca and P interactions as discussed above in practical diets compared to CVB-recommendations. These interactions were studied in two experiments in which the Ca and P content varied in diets with and without phytase, supplied to weaned pigs in a 4-5 week nursery period.

1.2 Objectives

The aim of this study was to determine the influence of dietary Ca and P inclusion level and Ca to P ratio on growth performance and faecal consistency, and digestibility and urinary excretion of Ca and P. In addition, the impact on skin damage due to (negative) behavioural interactions between the pigs was registered.

The following questions were addressed:

- What is the effect of a reduction in dietary Ca-content substantially below the CVB requirements for weaned pigs on animal performance, P utilisation and P-excretion in the urine.
- In case a reduction in dietary Ca content improves growth performance, to what extent is the negative effect of a higher dietary Ca content mediated by P-deficiency caused by an incremental dietary Ca content?
- How can P-utilisation be optimised without loss in growth performance and adequate bone integrity?
- What is the influence of microbial phytase on the interactions between Ca and P?

We hypothesised that practical diets for weaned pigs with reduced Ca content without reduction in P content would result in an oversupply of P and increased excretion of P in urine. Dietary P content in these diets could then be reduced without loss in performance. Secondly we hypothesised that a high dietary Ca content might have a negative impact of animal performance mediated via a reduction of P availability. The potential negative effect would then be negated by an increase in dietary P content

2 Material and Methods

The experiments described in this protocol are conducted because we are not aware of adequate (in vitro) models to study the in-vivo response of pigs to supplementation of dietary calcium and phosphorus. Moreover, the dietary treatments will not cause discomfort to the animals. Therefore, the study does not require permission from authorities for animal experiments (DEC, IVD). The study was conducted in research location Swine Nutrition Centre De Elsenpas by De Heus and a commercial pig farm equipped for applied research by ForFarmers.

2.1 Experimental animals

The study was conducted in group-housed newly weaned males and females pigs. At location De Heus a homogenous group of healthy pigs without history of disease and medication was selected, blocked on the basis of litter of origin, sex and body weight and randomly allocated to the treatments. The pigs were housed in pens of seven pigs, males and females separate, in two rooms with 32 pens each. At location ForFarmers groups of 11 representative littermates were allocated to the pens, hence littermates, males and females mixed, were kept together as an experimental unit. The study was conducted in two subsequent periods with 32 pens in four rooms per period. Details of the two locations are summarised in Table 1 below.

Table 1 *Summary of characteristics at the two study locations.*

	De Heus	ForFarmers
Animal species	Pigs	Pigs
Breeding line	Tempo growth boar x TN 70 sow	Topigs Pietrain x TN70
Status	Newly weaned pigs	Newly weaned pigs
Origin	Own breeding herd	Own breeding herd
Identification method	UHF transponder	Ear marks
Number and Gender	4 x 16 pens x 7 = 448 (about equal number of) male and female pigs	4 x 16 pens x 11 = 704 (about equal number of) male and female piglets
Age	Approx. 28 days of age	Approx. 26 days of age
Weight class	Weight varies form 3-11 kg which are normally blocked in 8 weight classes.	Littermates stay together at weaning
Health status	SPF status Vaccinations PRRS 5 days before weaning Circo: 2 weeks post weaning	Post weaning mortality <1% Incidental individual medication (<5% of piglets)

2.2 Treatments and design

The experiments were performed in research facilities of De Heus and ForFarmers with 4 treatments at each location. Each treatment group comprised 16 pens (experimental units) with 11 (ForFarmers) and 7 pigs (De Heus) per pen and in total 704 (ForFarmers) and 448 (De Heus) newly weaned pigs, respectively. Diets of Treatments 1 to 4 (De Heus) did not contain microbial phytase, diets of Treatments 5-8 (ForFarmers) were supplemented with 2000 FTU phytase per kg to replace an equivalent of mono calcium phosphate. The Ca and P content of the diets in each treatment is summarised below and included in Table 2.

T1/T5 Control diets, Ca and P content at levels of CVB-recommendations (referred to as high Ca with high Ca to P ratio: HCa, HCa/P);
T2/T6 Low Ca diets, Ca content based on practical use, P content at the level of control diets (referred to as low Ca with low Ca to P ratio: LCa, LCa/P);
T3/T7 Low Ca diets, Ca content based on practical use, low P content with resulting Ca/P ratio equal to control diets (referred to as low Ca with high Ca to P ratio: LCa, HCa/P);
T4/T8 High Ca diets, Ca content equal to control diet, increased P content with resulting Ca/P ratio equal to treatment 2 (referred to as high Ca with low Ca to P ratio: HCa, LCa/P).

Treatments 1 and 5 were reference diets based on CVB-recommendations without (T1) and with (T5) microbial phytase. Treatments 2 and 6 were included to determine whether a relatively low Ca content as often used in practical diets would have beneficial or negative effects on animal performance. Because a low Ca content might imply that pigs cannot optimally utilise all dietary P, Treatments 3 and 7 would indicate whether P content in such diets with low Ca content could be reduced without negative impact on the pigs. Because a potential beneficial effect of a low Ca content might be caused by a less negative effect on P digestibility, Treatments 4 and 8 were included to determine whether a potential negative effect of a high Ca content would be compensated by an increase in digestible P content. This would indicate that high Ca has a negative impact mediated by a reduced P digestibility.

The experiment will be analysed using the 2x2x2 factorial arrangement with study location/dietary phytase inclusion, Ca content and Ca/P ratio as respective factors. In Table 2 the experimental treatments have been arranged accordingly. The results will be presented in the same format.

Table 2 *Summary of dietary treatments and characteristics according to the factorial arrangement. Treatments 1-4 without microbial phytase, Treatments 5-8 with 2000 FTU/kg replacing mono calcium phosphate.*

Location, phytase	De Heus, no phytase				ForFarmers, with phytase			
Ca content	High Ca		Low Ca		High Ca		Low Ca	
Ca/P ratio	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P
Treatment	1	4	3	2	5	8	7	6
Phase 1 diets [1]								
EW (per kg)	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19
STTD P (g/kg)[2]	4.5	7.5	3.2	4.5	4.5	7.5	3.2	4.5
Ca (g/kg)	11.7	11.7	7.0	7.0	10.2	10.2	5.5	5.5
Ca from phytase	-	-	-	-	1.5	1.5	1.5	1.5
Dig. P from phytase	-	-	-	-	1.4	1.4	1.4	1.4
ratio Ca/STTD-P	2.6	1.55	2.2[3]	1.55	2.6	1.55	2.2[3]	1.55
Phase 2 diets								
EW (per kg)	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19
STTD P (g/kg)	3.8	5.4	3.0	3.8	3.8	5.4	3.0	3.8
Ca (g/kg)	10.0	10.0	7.0	7.0	8.5	8.5	5.5	5.5
Ca from phytase	-	-	-	-	1.5	1.5	1.5	1.5
Digestible P from phytase	-	-	-	-	1.4	1.4	1.4	1.4
ratio Ca/STTD-P	2.6	1.85	2.3[3]	1.85	2.6	1.85	2.3[3]	1.85

1) Experiment by De Heus without phytase, experiment by ForFarmers with 2000 FTU phytase per kg of diet.
2) The mean ATTD P content of the diets was 0.2 g/kg lower than STTD P content.
3) In pigs receiving diets with reduced Ca content the bone mineralisation is reduced and hence less P is required for bone formation. Retention of P in soft tissue (muscle, organ, skin) should not be reduced, therefor the adopted optimal Ca/P ratio in Treatment 3 and was lower than in Treatment 1 and 5.

2.3 Diets and feeding

In the suckling period the piglets received a commercial creep feed. From weaning, all pigs received the feeds according to the dietary treatments. Diets were based on requirements of weaned pigs in phase 1 and phase 2 nursery diets, apart from Ca and P (CVB, 2019). Feeds and drinking water were freely available during the experimental period. No acidification or medication of drinking water was applied during the trial. The composition of the basal diets was based on common feed ingredients (cereal grains, hipro soya, potato protein, whey product, soya oil), with low Ca-content. Diets were adequate in nutrients (energy, amino acids, vitamins and minerals) apart from Ca and P, for weaned pigs in this period and free of microbial phytase and other feed additives. Intrinsic phytase was minimised by expanding pelleting the basal diet. Thereafter, this mixture was ground and used as basis for the treatment diets, supplemented with micronutrients, the required amount of Ca, P, and phytase and again pelleted. Diet composition is included in Annex 1. The total copper content was 140 and 90 mg/kg and the total zinc content 137 and 92 mg/kg in phase 1 and phase 2 diets, respectively.

Experimental diets were produced by a feed production plant for research diets (ForFarmers, location Heijen) using a double mixing procedure to assure equal composition of the experimental diets. A basal diet was prepared, expander pelleted, ground, split into eight portions with equal composition, to which limestone was added by exchange with diamol and monocalcium phosphate (MCP) in exchange with NaCl and diamol. Titanium oxide (0.3%) was included as marker for digestibility. A phytase-free vitamin and mineral premix was included in all diets. All diets used by ForFarmers were supplemented with 2000 FTU phytase per kg. The diets were pelleted in 3 mm pellets. Samples of the diets were taken during feed production and analysed for Ca and P prior to the start of the experiment. Phytase was additionally analysed to verify correct inclusion and potentially remaining intrinsic phytase. Experimental diets were stored and delivered in labelled 20 kg bags or bulk according to the requirements of the study location.

In the suckling period the piglets received a commercial creep feed. From weaning, all pigs received one of the experimental diets:

- Phase 1 diets from weaning until 14 days post weaning
- Phase 2 diets from day 15 to end of the trial

2.4 Housing and management

At location De Heus the weaned pigs were housed in groups of 7 in pens of ca. 0.4 m² per pig, with partly slatted (60%) floors. One batch of piglets was housed in two rooms with 32 pens each. Each pen had a dry feeder with two feeding places and two drinking nipples. In addition, each pen was equipped with a rope and pigs received daily lucerne as enrichment material. Temperature and ventilation were in agreement with the requirements of pigs of this age: room temperature 26-28°C on Day 0 t/m 3 and 23°C thereafter. Rooms were mechanically ventilated and heated via floor heating and radiators (deltabuizen). Lights were on from 7.00 to 17.00h.
At location ForFarmers the weaned pigs were housed in groups of 11 piglets in pens of ca. 0.4 m² per pig, with partly slatted concrete floors. Each pen had a dry feeder with one feeding place and a drinking nipple. In addition an extra dry feeder was available to provide extra feeder space for the first week post weaning. Feeders were filled manually. In addition, each pen was equipped with a plastic tube on a chain as enrichment material. Temperature and ventilation were in agreement with the requirements of pigs of this age: room temperature >25°C on Day 0 t/m 3 and >23°C thereafter. Rooms were mechanically ventilated and heated via radiators on the wall. During the first two days post weaning there was continuous TL-light, followed by continuous 40 Lux light until the end of the nursery period.
Animals were daily monitored for health and (feeding) behaviour. All health problems and deviations from normal were registered. Medication was only allowed on individual basis according to the farm health and medication plan. For each treatment, date, pig, pen, diagnosis, medication and dose were registered. Pigs that did not recover after the appropriate medical treatment and would suffer from their illness were removed from the experiment. For culled and dead animals, date, pig, pen, likely cause of death and body weight were registered.

2.5 Observations

2.5.1 Performance and health

Incidence of diseases, required veterinary treatments, mortality, and any other adverse events were registered daily. Room temperature and ventilation rate were registered and stored in the climate computer or manually recorded as a backup to explain eventual incidences or deviations in the results of this study.
Individual body weight of the piglets was recorded on the day of weaning, Day 14 at diet change-over from pre-starter to starter diets, and on Day 28 or Day 35, end of the experimental period at location ForFarmers and De Heus, respectively. In addition, the pen weight was determined at location De Heus on Day 28 to allow determination of growth performance in the same periods in the two locations. Residual feed in the feeders was recorded on the days that the pigs were weighed as well. From these data, average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated for both locations for the periods Day 0-14, Day 14-28, and Day 0-28, and for Day 14-35 and Day 0-35 for location De Heus. Calculated ADFI and FCR were corrected for culled pigs.
Faecal consistency was recorded by the same panel of observers four times in the first 10 days (Day 4, 6, 8, and 10) on both locations. Thereafter, faecal consistency was registered on Day 17, 24, and 31 by De Heus and Day 12 and 21 by ForFarmers. Faecal consistency was registered on a pen-based scale: 0 = firm/normal faeces; 1 = soft faeces; 2 = thin faeces/diarrhoea; 3 = very thin faeces/watery diarrhoea; 9 = no faeces/no scoring possible. The pen value was used as input for the statistical analysis.

At the moments of individual weighing, 2 and 4/5 weeks post weaning, damage of ears, tail and flanks of the pigs was registered using a simplified scoring derived from an earlier study of Van der Peet et al. (2017) in three categories:

- Ears: 0 none; 1 mild damage/necrosis of edges; 2 serious damage/necrosis, wounds;
- Tail: 0 none; 1 bite marks; 2 visible injuries/wounds;
- Flanks: 0 none; 1 mild/small scratches, bruises <2 cm; 2 serious scratch/bruises >2 cm.

Based on the individual scoring multiplied by the number of pigs, a mean pen value was calculated and used as input for the statistical analysis.

2.5.2 Nutrient digestibility and urinary composition

Grab samples of fresh faeces (not contaminated by urine) were collected from a minimum of four pigs per pen twice daily during three successive days in the fourth week of the study. In this period the pigs received the starter diet with titanium as a marker to determine nutrient digestibility. Samples were pooled per pen for all three days and analysed. Apparent total tract digestibility coefficient ((ATTD, %) was calculated using the equation:

$$ATTD\ (\%) = 100 - 100 \times [nutrient]_{faeces} / [nutrient]_{feed} * [marker]_{feed} / [marker]_{faeces}$$

In the same three-day period, spot samples of urine were collected from as many pigs per pen as feasible. In the morning, while the rooms were still scarcely illuminated, a team of people quietly entered the room and spread out among the pens. When the lights were switched on, urine was collected by gently putting a small container mounted to a stick directly in the stream of urine from urinating pigs. Samples were pooled per pen for all three days and analysed for Ca and P content.

2.6 Description of analytical methods

The composition of the basal diets was analysed prior to use to verify Ca and P content and absence of intrinsic phytase. All prestarter and starter diets (n=16) were analysed for dry matter (DM), crude ash, Ca, P, titanium and phytase content using wet chemical analyses. Crude protein, crude fat, crude fibre, starch, and saccharose were analysed in all diets using NIR. The faecal samples were homogenised, oven-dried and analysed for DM, crude ash, Ca, P, and titanium. All feed and feacal samples were ground over a 1 mm sieve at 12000 rpm (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany) before analyses. Feed samples were ground without prior drying. Analytical methods were performed according to the International Organisation of Standardisation (ISO, Geneva, Switzerland) for the following components: dry matter (ISO 6496, 1999), ash (ISO 5984, 2002) and phytase (NEN-EN-ISO 30024). Feed and faecal samples were analysed in duplicate for all analytical methods. Subsequently, the Ca, P, and Ti content was determined using an ICP-OES (ThermoFisher, iCAP 7000) after destruction by a mixture of HCl (Chem-Lab), HNO_3 (Chem-lab) and HF (VWR) using a Microwave (CEM). Calcium and P content in urine were analysed using ICP-OES after sonication with ultrasound to reduce particle size of crystals, and subsequent destruction. A sample of drinking water was analysed for Ca using ICP-OES after destruction.

2.7 Statistics

Response variables are analysed by analysis of variance (ANOVA) using dietary treatment (factorial design) as fixed effect and room/block/animal as random effect, using GenStat (2020) statistical software.

The data were analysed with analysis of variance (ANOVA) as a randomised block design using Genstat statistical software. The pen was the experimental unit for the response parameters. The general model included dietary treatment as fixed effect in a factorial arrangement with Ca-content and Ca/P ratio as respective factors, and room and body weight block (replicate) within room as random effects:

$$Y_{ij} = \mu + Room_i + Block_j / Room_i + Calcium_k + Ca/P_l + Calcium_k \times Ca/P_l + e_{ijk}$$

in which:

Y_{ijk}	= dependent variable,
μ	= overall mean
Room	= room effect (i=1,2 De Heus and 1,… 8 ForFarmers)
$Block_i$	= block effect (j=1, 2, 3, 4 for each sex) within room (Location De Heus only)
$Calcium_k$	= effect of dietary Ca content (k= 1,2 representing low and high Ca)
Ca/P_l	= effect of dietary Ca/P (l= 1,2 representing low and high Ca/P)
e_{ijkl}	= residual error.

This model was used to analyse the results of the performance parameters (e.g. body weight, ADG, ADFI and FCR), faecal consistency, nutrient digestibility and urinary Ca and P concentration for each of the two study locations, separately. The scoring of skin, tail and ear damage of individual pigs was multiplied by the number of damaged pigs and divided by the number of pigs per pen. The resulting mean pen value was used as input for the statistical analysis using the same model. In addition, the data of the two locations were combined and analysed by inclusion of study location as random factor and phytase as fixed factor in the model. The effect of phytase was fully aliased with study location and could therefore not be determined. In contrast, the interactions between phytase, dietary Ca and Ca/P ratio were determined and presented. Thus, we assume that these interactions, i.e. potentially different effects of dietary Ca and Ca/P in phytase-free and phytase supplemented diets were large the results of the presence or absence of microbial phytase. Nonetheless, it should be realised that also other differences could play a role.

A Fisher protected t-test has been used for comparison of treatment means at an overall treatment effect of $P<0.1$. Pairwise differences are marked with superscripted indices when significant at $P<0.05$.

3 Results

3.1 General

The experiments are conducted according to the protocols without major deviations. Collection of urine and faeces samples was also successfully completed according to the protocol.

3.2 Health and medical treatments

3.2.1 Mortality and medical treatments

Overall, the pigs were in good health during the experimental period. At location De Heus, 12 pigs required medical treatments, the majority because of locomotion disorders. One pig was removed and euthanized because of a broken leg. At location ForFarmers, no medical treatments were required. Seven pigs were removed from the experiment during the first 14 days because of generally poor body condition and low body weight of these pigs in comparison to the pen mates.

Table 3 *Influence of dietary calcium content and calcium to phosphorus ratio in nursery diets on required medical treatments and mortality of weaned pigs during a 4-5-week experimental period[1].)*

Treatments	High Ca		Low Ca	
	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P
De Heus				
Initial number of pigs	112	112	112	112
Medicated piglets, n	3	5	3	1
Reasons for medication				
- Locomotion disorders	2	3	3	1
- Digestive disorders	1	1	-	-
- Miscellaneous	-	1	-	-
Mortality of piglets, n	1	-	-	-
Causes of death				
- Miscellaneous	1	-	-	-
ForFarmers				
Initial number of pigs	176	176	176	176
Medicated piglets, n	-	-	-	-
Mortality of piglets, n	1	-	-	-
Removed, wasting	2	2	1	2

1) Experiment by De Heus without phytase, experiment by ForFarmers with 2000 FTU phytase per kg of diet.

3.2.2 Occurrence of skin damage

In Table 4 the results of the registration of skin damage at the moment of individual weighing of pigs is summarised. The results were substantially different between the two locations. At location De Heus, no damage of flanks was observed, whereas a large number of pigs with tail damage was observed on Day 14 and 35, and a substantial number of pigs with ear damage on Day 14 but not on Day 35. The mean score for ear damage (i.e. the mean number of pigs per pen with ear damage) on Day 14 was lower (P=0.037) on low Ca diets. In contrast, on location ForFarmers, virtually no pigs with tail damage were observed, a small number with flank damage on Day 14 and a somewhat higher number of pigs with ear damage on Days 14 and 28. The reduction in Ca/P tended (P=0.072) to enhance the number of pigs with ear damage on Day 14. The reduction in Ca/P reduced the mean score for flank damage on Day 14 at a high Ca content, but it reduced this score at a low Ca content (interaction P=0.025). At location ForFarmers and to a lesser extent location De Heus, the number of pigs with ear damage was unevenly distributed among pens. A few pens had a high number of pigs with damaged ears and many pens had only a few or no pigs with ear damage. Tail damage was only observed at location De Heus, and more evenly distributed among pens.

Table 4 *Influence of dietary calcium content and calcium to phosphorus ratio in nursery diets on skin damage of weaned pigs at the moment of weighing on Day 14 and 28/35 during a 4-5-week experimental period[1,2,3].*

Treatments	High Ca		Low Ca		SEM	P-value[4]		Ca ×
	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P		Ca	Ca/P	Ca/P
De Heus	1	4	3	2				
Day 14 Ears								
- No of pens	11	9	10	4				
- No of pigs	28	20	16	7				
- Mean score[5]	0.25	0.18	0.14	0.06	0.052	0.037	0.151	0.932
Day 14 Tail								
- No of pens	16	16	16	16				
- No of pigs	75	81	77	67				
- Mean score	0.67	0.72	0.69	0.60	0.057	0.352	0.755	0.216
Day 35 Ears								
- No of pens	4	2	5	4				
- No of pigs	7	2	6	4				
- Mean score	0.06	0.02	0.05	0.04	0.020	0.823	0.121	0.502
Day 35 Tail								
- No of pens	16	16	15	15				
- No of pigs	59	58	60	58				
- Mean score	0.53	0.52	0.54	0.52	0.076	0.953	0.860	0.953
ForFarmers								
Day 14 Flank								
- No of pens	5	0	2	5				
- No of pigs	9	0	4	13				
- Mean score	0.05^{ab}	0.00^{a}	0.02^{ab}	0.07^{b}	0.022	0.310	1.000	0.025
Day 14 Ears								
- No of pens	6	6	4	7				
- No of pigs	7	20	8	17				
- Mean score	0.04	0.12	0.05	0.10	0.035	0.817	0.072	0.696
Day 14 Tail								
- No of pens	0	0	0	1				
- No of pigs	0	0	0	1				
- Mean score	0.00	0.00	0.00	0.01	0.003	0.322	0.322	0.322
Day 28 Ears								
- No of pens	1	2	3	1				
- No of pigs	1	4	16	6				
- Mean score	0.01	0.02	0.09	0.03	0.034	0.160	0.560	0.281

1) Experiment by De Heus without phytase, experiment by ForFarmers with 2000 FTU phytase per kg of diet.
2) Scoring of ears, tail and flanks: 0 none; 1 mild damage/bite marks/scratches; 2 serious damage/injuries/scratches.
3) All scores were 0 for flanks on Day 14 and 35 at location De Heus and flanks and tail on Day 35 at location ForFarmers
4) P-value, significance of treatment effects;
5) Mean score was calculated per pen as the sum of scores per pen divided by the number of pigs.

3.3 Growth performance

3.3.1 De Heus

The study was conducted in a five-week period from 8.73±0.54 to 26.1±3.09 kg body weight. The pigs realized a mean ADFI of 686 g/d, ADG of 495 g/d and FCR of 1.38. In the five week period, the lower dietary Ca content enhanced the ADG from 486 to 504 g/d (P=0.007), mediated by an increase in feed intake (P=0.027). The reduction in dietary Ca/P due to an increase in dietary P increased the FCR from 1.37 to 1.39 (P=0.036). This effect was mainly present in the high Ca diets as indicated by the interaction in FCR in the 28 d period. These tendencies in ADG and FCR were numerically (not significant) present in each of the two periods.

Table 5 *Influence of dietary calcium content and calcium to phosphorus ratio on growth performance of weaned pigs during a 5-week experimental period1).*

Treatments	High Ca		Low Ca		SEM2)	P-value3)		
	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P		Ca	Ca/P	Ca × Ca/P
Treatment	1	4	3	2				
Body weight, kg								
Day 0	8.75	8.63	8.76	8.77	0.075	0.323	0.460	0.418
SD BW	0.56	0.49	0.52	0.58	0.034	0.515	0.875	0.070
Day 14	12.62	12.41	12.73	12.73	0.096	0.032	0.297	0.278
SD BW	1.26	1.05	1.19	1.12	0.088	0.967	0.122	0.438
Day 28 4)	20.26	20.04	20.33	20.58	0.178	0.096	0.907	0.190
Day 35	25.82	25.62	26.32	26.50	0.218	0.003	0.965	0.372
SD BW	3.09	2.99	3.21	3.08	0.193	0.581	0.562	0.961
Day 1-14								
Body gain, g/d	276	270	283	283	6.8	0.151	0.630	0.639
Feed intake, g/d	362	367	377	374	7.1	0.149	0.930	0.570
FCR	1.31	1.36	1.33	1.33	0.022	0.660	0.343	0.184
Day 14-28								
Body gain, g/d	546	545	543	561	10.0	0.525	0.385	0.352
Feed intake, g/d	756	771	778	798	13.8	0.081	0.214	0.866
FCR	1.39	1.42	1.43	1.43	0.014	0.073	0.509	0.205
Day 14-35								
Body gain, g/d	629	629	647	656	8.5	0.010	0.610	0.613
Feed intake, g/d	873	893	899	919	11.9	0.033	0.109	0.988
FCR	1.39	1.42	1.39	1.40	0.012	0.393	0.103	0.518
Day 1-28								
Body gain, g/d	411	408	413	422	6.7	0.229	0.684	0.351
Feed intake, g/d	559	569	577	586	8.6	0.049	0.304	0.920
FCR	1.36a	1.40b	1.40b	1.39ab	0.010	0.198	0.175	0.026
Day 1-35								
Body gain, g/d	488	485	501	507	6.3	0.007	0.835	0.539
Feed intake, g/d	669	682	690	701	8.7	0.027	0.176	0.862
FCR	1.37	1.41	1.38	1.38	0.009	0.312	0.036	0.143

1) Experiment by De Heus with phytase-free diets
2) SEM, pooled standard error of the means.
3) P-value, significance of treatment effects.
4) Based on pen weight, no SD available

3.3.2 ForFarmers

The study was conducted in a four-week period from 7.68±1.03 to 17.11±2.19 kg body weight. The pigs realized a mean ADFI of 468 g/d, ADG of 337 g/d and FCR of 1.40. In the four-week period, the lower dietary Ca content tended to enhance the ADFI from 459 to 478 g/d (P=0.078) without significant effect on ADG. The reduction in dietary Ca/P due to an increase in dietary P increased the FCR from 1.37 to 1.42 (P=0.011). This effect was mainly present in the high Ca diets as indicated by the interaction in FCR in the 28 d period. These tendencies in ADG and FCR were numerically (not significant) present in each of the two periods.

Table 6 *Influence of dietary calcium content and calcium to phosphorus ratio on growth performance of weaned pigs during a 4-week experimental period[1)]*

Treatments	High Ca		Low Ca		SEM[2)]	P-value[3)]		
	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P		Ca	Ca/P	Ca × Ca/P
Treatment	5	8	7	6				
Body weight, kg								
Day 0	7.56	7.63	7.69	7.85	0.207	0.407	0.573	0.826
SD BW	0.94	1.05	0.96	1.15	0.068	0.389	0.037	0.554
Day 14	10.01	10.04	10.31	10.36	0.235	0.196	0.855	0.973
SD BW	1.21	1.35	1.19	1.43	0.088	0.735	0.042	0.562
Day 28	16.89	16.94	17.42	17.17	0.342	0.270	0.776	0.662
SD BW	1.99	2.26	2.05	2.45	0.154	0.425	0.032	0.665
Day 1-14								
Body gain, g/d	175	172	187	179	6.0	0.118	0.383	0.654
Feed intake, g/d	240	255	259	263	8.6	0.124	0.286	0.529
FCR	1.38	1.49	1.40	1.47	0.031	0.970	0.004	0.470
Day 14-28								
Body gain, g/d	492	493	508	487	13.1	0.691	0.445	0.393
Feed intake, g/d	660	682	700	688	13.9	0.096	0.703	0.231
FCR	1.35	1.39	1.38	1.42	0.024	0.237	0.097	0.978
Day 1-28								
Body gain, g/d	333	340	348	333	8.2	0.368	0.351	0.395
Feed intake, g/d	450	468	479	476	10.3	0.078	0.482	0.284
FCR	1.36	1.42	1.38	1.43	0.021	0.315	0.011	0.771

1) Experiment by ForFarmers with 2000 FTU phytase per kg of diet.
2) SEM, pooled standard error of the means.
3) P-value, significance of treatment effects.

3.3.3 Combined results

In Appendix 3, combined results and analyses of the two studies are presented. The analyses includes possible interactions due to the inclusion of phytase in the experiment conducted by ForFarmers. The overall effect of phytase could not be tested since this is completely aliased with the two study locations. In the 28 d period the lower dietary Ca content enhanced the ADFI from 512 to 530 g/d (P=0.009). This increase in ADFI enhanced the ADG from day 1-14, but not from day 14-28. From day 14-28 the reduction in Ca content tended to increase the FCR (P=0.056) but this effect was not significant in the overall period. The reduction in Ca/P due to an increase in dietary P increased the FCR from 1.37 to 1.41 (P=0.005). This effect tended to be more prominent in the phytase supplemented diets as indicated by the interaction (P=0.082). The effect of Ca/P on FCR was present in each of the two periods, but most prominent from d 1-14 in phytase supplemented diets with FCR is 1.39 and 1.48 for high and low Ca/P diets.

3.4 Feacal consistency

3.4.1 De Heus

The faecal consistency score ranged from 0 (firm) to 3 (diarrhoea). The mean faecal consistency score decreased from 1.15 on Day 4 to 0.89 on Day 6, remained stable until Day 17, increased to 1.30 on Day 24 and decreased again to 0.89 on Day 31. The dietary treatments did not significantly affect faecal consistency in the first two weeks when pigs received the weaning diet. On Day 24 the low Ca/P reduced the faecal score (i.e. firmer faeces) in the low Ca diet, but not in the high Ca diet (Interaction P=0.042). On Day 31 high dietary Ca enhanced the faecal score (i.e. softer faeces) (P=0.037). The mean faecal dry matter content in faeces samples, used to determine nutrient digestibility, was 261 g/kg and not affected by dietary treatments.

Table 7 *Influence of dietary calcium content and calcium to phosphorus ratio on consistency and dry matter content of faeces of weaned pigs during a 5-week experimental period[1)]*

Treatments	High Ca		Low Ca		SEM[2)]	P-value[3)]		
	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P		Ca	Ca/P	Ca × Ca/P
Treatment	1	4	3	2				
Faecal consistency[4)]								
Day 4	1.25	0.86	1.31	1.19	0.260	0.446	0.318	0.600
Day 6	1.06	0.56	0.94	1.00	0.210	0.461	0.304	0.188
Day 8	0.88	0.69	0.94	1.00	0.150	0.217	0.678	0.408
Day 10	0.88	0.94	1.00	0.81	0.100	1.000	0.535	0.217
Day 17	0.88	1.00	0.94	0.94	0.044	0.950	0.146	0.146
Day 24	1.19ab	1.38ab	1.62b	1.00a	0.200	0.929	0.253	0.042
Day 31	1.00	1.06	0.69	0.81	0.131	0.037	0.477	0.812
Faecal dry matter, g/kg								
Day 23-24	259.8	262.4	261.8	260.0	3.85	0.961	0.924	0.576

1) Experiment by De Heus with phytase-free diets.
2) SEM, pooled standard error of the means.
3) P-value, significance of treatment effects.
4) Faecal score: 0 = firm/normal faeces; 1 = soft faeces; 2 = thin faeces/diarrhoea; 3 = very thin faeces/watery diarrhoea.

3.4.2 ForFarmers

The mean faecal consistency score decreased, from 0.92 and 1.03 on Day 4 and 6, respectively to 0.06 on Day 21, indicating the development of firmer faeces over time, with some fluctuations on intermediate days. The high dietary Ca content tended to increase the faecal consistency score (i.e. caused softer faeces) on Day 6 (P=0.095) whereas the low Ca/P tended to cause softer faeces on Day 10. (P=0.075). The mean faecal dry matter content in faeces samples to determine nutrient digestibility was 260 g/kg and reduced for pigs receiving the high Ca diet with low Ca/P ratio (i.e. high P content) (Interaction P=0.008).

Table 8 *Influence of dietary calcium content and calcium to phosphorus ratio on consistency and dry matter content of faeces of weaned pigs during a 4-week experimental period[1].)*

Treatments	High Ca		Low Ca		SEM[2)]	P-value[3)]		
	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P		Ca	Ca/P	Ca × Ca/P
Treatment	5	8	7	6				
Faecal consistency[4)]								
Day 4	1.06	0.88	0.94	0.81	0.234	0.691	0.508	0.894
Day 6	1.31	1.19	0.88	0.75	0.257	0.095	0.629	1.000
Day 8	0.25	0.50	0.44	0.31	0.158	1.000	0.732	0.306
Day 10	0.69ab	0.81ab	0.38a	0.88b	0.172	0.471	0.075	0.281
Day 12	0.38	0.31	0.06	0.50	0.163	0.704	0.256	0.132
Day 21	0.00	0.00	0.00	0.12	0.062	0.322	0.322	0.322
Faecal dry matter, g/kg								
Day 25-27 ?	263.2b	246.2a	262.4b	266.7b	3.85	0.013	0.106	0.008

1) Experiment by ForFarmers with 2000 FTU phytase per kg of diet.
2) SEM, pooled standard error of the means.
3) P-value, significance of treatment effects.
4) Faecal score: 0 = firm/normal faeces; 1 = soft faeces; 2 = thin faeces/diarrhoea; 3 = very thin faeces/watery diarrhoea;

3.4.3 Combined results

In Appendix 4, combined results and analyses of the faecal consistency on Day 4 to 10, determined in each of the two studies are presented. The analyses includes possible interactions due to the inclusion of phytase in the experimental diets. No major effects of dietary treatments were observed. On Day 6 the low Ca content tended to reduce the faecal consistency score in phytase supplemented diets but not in phytase-free diets (Interaction P=0.079). On Day 10, the low Ca/P content tended to increase the faecal consistency score in phytase supplemented diets but not in phytase-free diets (Interaction P=0.068). The mean faecal dry matter content was affected by dietary Ca and Ca/P in a three way interaction (P=0.021), caused by a lower dry matter content for pigs receiving a phytase supplemented diet with a high Ca content and a low Ca/P ratio due to an increase in P supplementation.

3.5 Nutrient digestibility

3.5.1 De Heus

The nutrient digestibility as determined by grab sampling of faeces on Day 23-25 of the experimental period is presented in Table 9. The low dietary Ca content increased the ATTD of organic matter from 80.3 to 81.5% (P=0.010), the ATTD of Ca from 47.0 to 55.3% (P<0.001) and tended to reduce the ATTD of P from 45.4 to 43.2% (P=0.086). The reduction in Ca/P with increasing dietary P enhanced the ATTD of P from 41.0 to 47.6% (P<0.001).

Table 9 *Influence of dietary calcium content and calcium to phosphorus ratio on total tract nutrient digestibility in weaned pigs in week 4 after weaning[1)].*

Treatments	High Ca High Ca/P	Low Ca/P	Low Ca High Ca/P	Low Ca/P	SEM[2)]	P-value[3)] Ca	Ca/P	Ca × Ca/P
Treatment	1	4	3	2				
Digestibility, %								
Dry matter	76.92	77.99	78.08	78.25	0.503	0.162	0.226	0.376
Organic matter	79.93	80.64	81.50	81.43	0.441	0.010	0.475	0.379
Ash	42.56	47.96	40.60	43.96	1.259	0.022	0.001	0.422
Calcium	46.58	47.45	55.47	55.21	0.990	<0.001	0.758	0.569
Phosphorous	42.63	48.07	39.32	47.16	1.201	0.086	<0.001	0.322

1) Experiment by De Heus with phytase-free diets.

2) SEM, pooled standard error of the means.

3) P-value, significance of treatment effects.

3.5.2 ForFarmers

The nutrient digestibility as determined over the last three days of the experimental period is presented in Table 10. The low dietary Ca content increased the ATTD of organic matter from 82.4 to 83.6%, but only for the low Ca/P, i.e. diets with a relatively high P content (Interaction P=0.006). The reduction in Ca content enhanced the ATTD of Ca from 63.8 to 74.6% (P<0.001) and enhanced the ATTD of P from 69.5 to 71.5% (P=0.009). The reduction in Ca/P with increasing dietary P tended to reduce the ATTD of Ca from 70.2 to 68.2 (P=0.052) and enhanced the ATTD of P from 69.2 to 71.9% (P<0.001).

Table 10 *Influence of dietary calcium content and calcium to phosphorus ratio on total tract nutrient digestibility in weaned pigs in week 4 after weaning[1)].*

Treatments	High Ca High Ca/P	Low Ca/P	Low Ca High Ca/P	Low Ca/P	SEM[2)]	P-value[3)] Ca	Ca/P	Ca × Ca/P
Treatment	5	8	7	6				
Digestibility								
Dry matter	80.35	79.96	79.99	80.52	0.223	0.644	0.746	0.043
Organic matter	83.13b	82.40a	83.08b	83.58b	0.212	0.010	0.572	0.006
Ash	49.85b	53.41c	46.91a	47.14a	0.648	<0.001	0.005	0.013
Calcium	64.84	62.77	75.62	73.53	1.047	<0.001	0.052	0.990
Phosphorous	67.80	71.18	70.55	72.52	0.749	0.009	<0.001	0.351

1) Experiment by ForFarmers with 2000 FTU phytase per kg of diet.

2) SEM, pooled standard error of the means.

3) P-value, significance of treatment effects.

3.5.3 Combined results

In Appendix 5, combined results and analyses of the total tract nutrient digestibility, determined in each of the two studies, are presented. Overall, the ATTD was substantially higher on the location with phytase included in the diets: +2% for OM, +18% for Ca and +33% for P. The reduction in Ca content increased the ATTD of OM (P<0.001), but this effect was dependent on phytase and Ca/P in the diet (Interaction P=0.038). The reduction in dietary Ca content enhanced the ATTD of Ca (P<0.001) from 55.4 to 65.0% with a somewhat greater effect in phytase supplemented than phytase-free diets (Interaction P=0.094). The reduction in Ca/P tended to reduce the ATTD of Ca but only in phytase supplemented diets (Interaction P=0.102). The ATTD of P was enhanced by a reduction in Ca/P by increasing P from 41.0 to 47.6 in phytase-free diets and from 69.2 to 71.9 in phytase-supplemented diets (Interaction P=0.005). The reduction in Ca reduced ATTD of P in phytase-free diets and enhanced the P digestibility in phytase supplemented diets (Interaction P=0.004).

3.6 Realised digestible calcium and phosphorus in diets

To interpret the results of this study, it is important to determine the amount of dietary Ca and P that was actually digested by the pigs and available for body gain and bone mineralisation. Based on the analysed diet composition and the realised digestibility as discussed in the previous paragraph, Table 11 provides both the calculated and actually realised digestible Ca and P content.

In all phytase-free diets, the digestibility of P was lower than the calculated values. In Treatment 2 the difference was approximately 7% points, in the other three treatments 10-12% points. The STTD of Ca was close to the adopted 60% in the two low Ca diets (Treatments 2 and 3) but 10% points lower on Treatments 1 and 4 with high Ca diets. As a result of the lower digestibility of both Ca and P, the ratio between digestible Ca and P was close to expected in Treatments 1 and 4, but higher in Treatments 2 and 3. In the phytase supplemented diets, the determined P and Ca digestibility were similar to the calculated values in the low Ca diets (Treatments 6 and 7) and 3-5% points lower in the high Ca diets (Treatments 5 and 8). In all phytase-supplemented diets, the ratio between digestible Ca and P was somewhat higher than expected, due to the higher analysed versus calculated Ca content in these diets. For this reason, the digestible Ca content was also higher than calculated in the low Ca phytase-supplemented diets.

Table 11 *Calculated and analysed digestible nutrient content (g/kg) of phase 2 (starter) diets for weaned pigs, based on analysed nutrient content and determined total tract nutrient digestibility.*

Location, phytase	De Heus, no phytase				ForFarmers, with phytase			
Ca content	High Ca		Low Ca		High Ca		Low Ca	
Ca/P ratio	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P
Treatment	1	4	3	2	5	8	7	6
Phase 2 (starter)								
Calculated[1)]								
Phosphorus, g/kg	6.8	8.7	5.8	6.8	5.1	7.0	4.1	5.1
STTD-P, g/kg	3.8	5.4	3.0	3.8	3.8	5.4	3.0	3.8
STTD-P, %	56.3	62.2	51.8	56.3	75.0	77.2	73.1	75.0
ATTD-P, g/kg	3.6	5.2	2.8	3.6	3.6	5.2	2.8	3.6
ATTD-P, %	53.7	60.2	48.8	53.7	71.5	74.7	68.8	71.5
Calcium, g/kg	10.0	10.0	7.0	7.0	8.5	8.5	5.5	5.5
STTD Ca, g/kg	6.0	6.0	4.2	4.2	6.0	6.0	4.2	4.2
STTD-Ca, %	60.0	60.0	60.0	60.0	70.6	70.6	76.4	76.4
ATTD-Ca, g/kg	5.8	5.8	4.0	4.0	5.8	5.8	4.0	4.0
ATTD-Ca, %	57.8	57.8	56.9	56.9	68.0	68.0	72.4	72.4
STTD Ca/P	1.58	1.11	1.40	1.11	1.58	1.11	1.40	1.11
ATTD Ca/P	1.59	1.11	1.41	1.10	1.59	1.11	1.41	1.10
Analysed								
Phosphorus, g/kg	6.9	8.8	5.7	6.9	5.2	7.1	4.3	5.2
STTD-P, g/kg	3.1	4.4	2.4	3.4	3.7	5.2	3.2	3.9
STTD-P, %	45.2	50.1	42.4	49.7	71.2	73.7	74.6	75.9
ATTD-P, g/kg	2.9	4.2	2.2	3.2	3.5	5.0	3.0	3.8
ATTD-P, %	42.6	48.1	39.3	47.2	67.8	71.2	70.6	72.5
Calcium, g/kg	10.1	10.1	7.3	7.3	9.2	9.2	6.4	6.4
STTD-Ca, g/kg	4.9	5.0	4.3	4.3	6.2	6.0	5.1	4.9
STTD-Ca, %	48.8	49.6	58.5	58.2	67.2	65.2	79.1	77.0
ATTD-Ca, g/kg	4.7	4.8	4.1	4.1	6.0	5.8	4.8	4.7
ATTD-Ca, %	46.6	47.5	55.5	55.2	64.8	62.8	75.6	73.5
STTD Ca/P	1.59	1.14	1.77	1.25	1.68	1.15	1.58	1.26
ATTD Ca/P	1.61	1.14	1.81	1.25	1.70	1.14	1.59	1.26

1) ATTD and STTD of P in phytase-free diets based on P digestibility of ingredients according to CVB (2019); ATTD and STTD of Ca based on the adopted value of 60% (Bikker and Blok, 2017). Digestibility values in phytase-supplemented diets based on the adopted contribution of 2000 FTU microbial phytase (see Table 2).

3.7 Urinary excretion

3.7.1 De Heus

The urinary content of Ca and P and their ratio were significantly affected by dietary treatments (Table 12). The urinary Ca content was drastically reduced by a decrease in Ca/P ($P<0.001$). The reduction in Ca content tended to increase the urinary Ca at the high Ca/P but not at the low Ca/P (Interaction $P=0.037$). Urinary P content was drastically enhanced by the reduction in Ca/P by increasing dietary P whereas the reduction in Ca reduced the urinary P content, but only for the low Ca/P diets (Interaction

P<0.001). The Ca/P in the urine was drastically reduced by a reduction in Ca/P in the diet. The reduction in Ca enhanced the Ca/P but only in the high Ca/P diet (Interaction P=0.076).

Table 12 *Influence of dietary calcium content and calcium to phosphorus ratio on excretion of calcium and phosphorous in the urine of weaned pigs in week 4 after weaning[1)].*

Treatments	High Ca		Low Ca		SEM[2)]	P-value[3)]		
	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P		Ca	Ca/P	Ca × Ca/P
Treatment	1	4	3	2				
Urinary content, mg/kg								
Calcium	557b	159a	885c	127a	83.6	0.083	<0.001	0.037
Phosphorous	226a	2072c	67a	948b	106.0	<0.001	<0.001	<0.001
Ca/P ratio	9.0b	-0.2a	17.2c	0.4a	2.10	0.042	<0.001	0.076

1) Experiment by De Heus with phytase-free diets.

2) SEM, pooled standard error of the means.

3) P-value, significance of treatment effects.

3.7.2 ForFarmers

The urinary content of Ca and the the Ca/P were significantly reduced by a lower dietary Ca/P but not affected by dietary Ca (Table 13). The urinary Ca content and Ca/P were drastically reduced by a decrease in Ca/P (P<0.001). Urinary P content was drastically enhanced by the reduction in Ca/P by increasing dietary P whereas the reduction in Ca reduced the urinary P content, but only for the low Ca/P diets.

Table 13 *Influence of dietary calcium content and calcium to phosphorus ratio on excretion of calcium and phosphorous in the urine of weaned pigs in week 4 after weaning[1)].*

Treatments	High Ca		Low Ca		SEM[2)]	P-value[3)]		
	High Ca/P	Low Ca/P	High Ca/P	Low Ca/P		Ca	Ca/P	Ca × Ca/P
Treatment	5	8	7	6				
Urinary content, mg/kg								
Calcium	1152	267	1393	302	150.8	0.364	<0.001	0.497
Phosphorous	12a	1066c	12a	283b	73.5	<0.001	<0.001	<0.001
Ca/P ratio	96.9	0.3	110.5	4.1	5.99	0.151	<0.001	0.415

1) Experiment by ForFarmers with 2000 FTU phytase per kg of diet.

2) SEM, pooled standard error of the means.

3) P-value, significance of treatment effects.

3.7.3 Combined results

The urinary content of Ca and P and their ratio were significantly affected by dietary treatments (Appendix 6). Numerically, the urinary Ca concentration and Ca/P were substantially higher and P concentration was lower in phytase supplemented diets. The urinary Ca content was drastically reduced by a decrease in Ca/P (P<0.001). This effect was greater in phytase supplemented diets than phytase-free diets (Interaction P=0.023) due to high urine Ca concentration for the phytase supplemented diet with low Ca/P. The urinary P concentration was drastically enhanced by the reduction in Ca/P by increasing dietary P. This effect was greater in phytase-free than in phytase supplemented diets and in high Ca compared to low Ca diets (Interaction P<0.001). The reduction in Ca reduced the urinary P content, but only for the low Ca/P diets (Interaction P<0.001). The Ca/P in the urine was drastically reduced by a reduction in Ca/P in the diet. This effect was greater in phytase-supplemented than in phytase-free diets due to the high Ca/P in urine of pigs with phytase supplemented diets with a high Ca/P.

4 Discussion

This study was conducted to determine the influence of dietary Ca and P content and Ca to P ratio on growth performance, digestibility of Ca and P and their urinary excretion. The following questions were addressed:

- What is the effect of a reduction in dietary Ca-content substantially below the CVB requirements for weaned pigs on animal performance, P utilisation and excretion in the urine, faecal consistency and skin damage caused by negative behaviour?
- In case a reduction in dietary Ca content improves growth performance, to what extent is the negative effect of a higher dietary Ca content mediated by P-deficiency caused by an incremental dietary Ca content?
- What is the influence of microbial phytase on the interactions between Ca and P
- How can P-utilisation be optimised without loss in growth performance and bone integrity?

We hypothesised that practical diets for weaned pigs with reduced Ca content without reduction in P content (thus reduced Ca/P) would result in an oversupply of P and increased excretion of P in urine. Dietary P content in these diets could then be reduced without loss in performance. Secondly we hypothesised that a high dietary Ca content might have a negative impact on animal performance mediated via a high buffering capacity or a reduction of P availability. In the latter case, the potential negative effect would then be negated by an increase in dietary P content.

The results briefly indicated that a reduction in Ca content and Ca/P as in practical diets enhanced ADFI in both phytase-free and phytase-supplemented diets, but enhanced ADG in phytase-free diets only. Indeed the urinary P content was enhanced by this Ca reduction and reduced when dietary P was reduced in the same ratio as dietary Ca. This reduction in dietary P did not reduce growth performance but enhanced the urinary Ca content suggestion an optimum ratio between the low and high Ca/P ratio. The lower growth rate at a high dietary Ca content, in fact only observed in phytase-free diets, was not improved by an increase in dietary P content. Hence, it was likely not mediated by a P deficiency, despite the observed negative effect of high dietary Ca content digestibility of P.

We will discuss results in more detail, starting with the nutrient digestibility and realised digestible dietary Ca and P content and their ratio, to use this information for the interpretation of the growth performance and practical recommendations.

4.1 Nutrient digestibility

4.1.1 Phytase

Overall, the nutrient digestibility was substantially higher for the phytase-supplemented diets than for the phytase-free diets. For the phytase-supplemented diets, the ATTD was higher by approximately 2% for organic matter, 18% for Ca and 33% for P. Although differences between the two locations may play a role, it is likely that the majority of this difference was caused by the inclusion of 2000 FTU/kg of microbial phytase and simultaneous reduction in dietary Ca and P content. This was in line with results of numerous studies that demonstrate the effect of microbial phytase on Ca and P digestibility (Kuhn and Manner, 2012; González-Vega et al., 2013; Bikker et al., 2021; Hu et al. 2021). Especially for Ca the simultaneous reduction in Ca content presumably made a large contribution to the enhanced digestibility as discussed below.

4.1.2 Proximate nutrients

The reduction in dietary Ca content substantially enhanced the digestibility of organic matter (+0.9%) in all diets apart from the phytase-supplemented diet with a high Ca/P. We suggest that buffering capacity of Ca, thus hampering the pH drop and the formation of Ca soaps may play a role. In an earlier study in growing pigs (30-45 kg) (Hu et al., 2021) we also observed a tendency for a reduction in ATTD of organic matter and a significant reduction in ATTD of crude fat with incremental dietary Ca from 6 to 10 g/kg, but not from 2 to 6 g/kg. In a study into limestone particle size in growing pigs (30-50 kg) we did not observe an effect of Ca content (8 vs 2 g/kg) on digestibility of organic matter and crude fat (Bikker et al., 2021). In conclusion, a high dietary Ca content may hamper digestibility of organic matter, but this effect seems relatively small at practical inclusion levels.

4.1.3 Calcium

The ATTD of Ca was enhanced by the reduction in Ca content. This effect was somewhat higher in phytase-supplemented diets (+11%) then in phytase-free diets (+8%). The increase in ATTD of Ca when dietary Ca was reduced suggests an upregulation of active absorption. This observation may indicate that inclusion of phytase enhanced ATTD of Ca not only by the release of Ca from degradation of phytate complexes but also indirectly by the reduction in dietary Ca content. A beneficial effect of Ca reduction on ATTD of Ca was also observed in phytase-supplemented diets in our previous study, but not in phytase-free diets (Hu et al., 2021). Similarly, Stein et al. (2011) did not observe a significant effect of dietary Ca content from limestone in phytase-free diets on ATTD of Ca. Presumably the net effect of Ca content on Ca ATTD of Ca depends on the ATTD of the basal diet and the supplement, as well as the Ca supply relative to the requirements. The Ca/P ratio, i.e. the P content at a constant Ca content did not affect the ATTD of Ca. This is in agreement with our earlier study in weaned pigs in the nursery period in which a reduction of dietary P content by 50% did not affect ATTD of Ca (Bikker et al., 2018). In conclusion, a reduction in dietary Ca content may enhance Ca digestibility.

4.1.4 Phosphorus

Overall, the dietary Ca content did not affect the ATTD of P. Nonetheless, in phytase-free diets the reduction in Ca content reduced the ATTD of P by 2% whereas in the phytase-supplemented diet the reduction in Ca content enhanced the ATTD of P. This observed interaction is partially in line with our earlier study in which a reduction in dietary Ca improved ATTD of P more in the presence of phytase. (Hu et al., 2021). The reduction in ATTD of P with a reduction in dietary Ca most likely was the result of the simultaneous reduction in dietary P from MCP with a higher digestibility than the phytase-free basal diet. This is supported by the larger increase in ATTD of P in phytase-free diets with the reduction in Ca/P realised by supplementation with MCP, than in phytase-supplemented diets. Also in this case, the ATTD of P from MCP was likely higher than that of the high Ca/P diets (41.0%), therefore the MCP supplement enhanced the ATTD of P. The ATTD of the high Ca/P phytase-supplemented diets was 69.2%, therefore, supplementary P from MCP only marginally increased the ATTD of P in this diet. The effect of a reduction in dietary Ca at constant dietary P content (practical diets versus CVB recommendations) can be derived from treatments 2 versus 1 and 6 versus 5. In this case the reduction in Ca content enhanced the ATTD of P by approximately 5% in both phytase-free and phytase-supplemented diets. This effect of dietary Ca content at constant dietary P content, thus reducing the Ca/P, on ATTD of P is in line with previous studies (Stein et al., 2011; Hu et al., 2021). These results indicate that a reduction in Ca content at constant P content enhanced the ATTD of P and the reduction of Ca content at constant Ca/P ratio reduced the ATTD of P in phytase-free diets because of the simultaneous reduction of P from MCP. A reduction in Ca/P due to an increase in P content from MCP enhanced the ATTD of P, in particular in phytase-free diets. In conclusion, a reduction in dietary Ca/P ratio by a lower Ca supplementation will likely improve P digestibility, whereas a reduction in Ca content may have less effect on digestibility of P.

4.1.5 Realised digestible calcium and phosphorus in diets

In all phytase-free diets, the digestibility of P was lower than the calculated values. Many reasons can be involved. In some treatments the lower digestion can be partly caused by the relatively high Ca content. In addition, table values of P digestibility have been determined at marginal dietary P contents. Hence, the inclusion of P close to (Treatments 1 and 2) or above (Treatment 4) requirements may have contributed to a lower digestibility. The age of the pigs in the present study was lower than the growing-finishing pigs used to determine table values. Results of Kemme et al. (1997) suggest that P digestibility may be slightly lower in 30 kg pigs compared to older pigs, but we are not aware of a direct comparison between weaned pigs and growing-finishing pigs. Finally, mean

table values of P digestibility in feed materials may not have been fully representative for the batches of ingredients used in the present study. The Dutch CVB has not yet adopted a system for Ca digestibility of feed materials. Therefore, a mean STTD of Ca of 60% in phytase-free diets was adopted to calculate the required total dietary Ca from the requirement at post absorptive level (Bikker and Blok, 2017). The results of the present study indicate that this was indeed about realised value at the low Ca diets, but not at high Ca diets.
In the phytase supplemented diets (2000 FTU/kg), the determined P and Ca digestibility were similar to the calculated values in the low Ca diets and 3-5% points lower in the high Ca diets. Although it is not possible to draw general conclusions about the differences between table values and realised digestibility based on this specific study, these results may suggest that the use of microbial phytase can reduce the risk of substantial deviations between assumed and actual dietary digestible Ca and P contents. Obviously, this would also depend on the efficacy and inclusion level of microbial phytase.

In summary, the realised digestible P content of the diets was in line with calculated values in the phytase supplemented diets, but overestimated in the phytase-free diets. The realised digestible Ca content of the diets was in line with calculated values in the low Ca diets and slightly or substantially overestimated in the high Ca phytase supplemented and phytase-free diets, respectively. Because of differences between calculated and analysed total content and digestibility of Ca and P, we will include the analysed values in the discussion and interpretation in following paragraphs.

4.2 Urinary excretion of Ca and P

4.2.1 Sampling

We analysed the Ca and P concentration in spot samples of urine collected directly during urination of pigs. Influences of dietary treatments on urinary concentration reflect changes in excretion, assuming that the volume of urine is not substantially affected. In any case the ratio between Ca and P concentration is indicative since this is not affected by the volume of urine. Nonetheless, values of the Ca/P ratio are highly variable due to the influence of (very) low P values that can drastically enhance the Ca/P ratio.

4.2.2 Influence of calcium content and calcium to phosphorus ratio

Both the Ca and P concentration were primarily affected by the dietary Ca/P (Appendix 6). A reduction in dietary Ca/P realised by an increase in P from MCP enhanced the P excretion and reduced the Ca excretion and the urinary Ca/P ratio. This indicates that with incremental P, more absorbed Ca could be retained in bone and hence less was excreted in urine. The substantial increase in urinary P indicates a relatively oversupply of P. The increase in P excretion was bigger in phytase-free diets, suggesting that Ca limited the retention of P more in these diets than in the phytase-supplemented diets. This is in line with the high Ca excretion in phytase supplemented high Ca diets (Treatments 5 and 7) that was drastically reduced with the reduction in Ca/P (Treatments 6 and 8).

An increase in Ca content at constant Ca/P enhanced the P excretion more than the Ca excretion and thus reduced the Ca/P in the urine. This effect of increasing Ca content was only present at the low Ca/P (Treatment 2 vs 4 and 6 vs 8) but not at high Ca/P (Treatment 3 vs 1 and 7 vs 5) as indicated by the interaction between Ca and Ca/P on urinary P content. The reason is that with increasing Ca content the increase in calculated STTP P content was 1.6 g/kg at the low Ca/P and 0.8 at the high Ca/P, while the increase in calculated STTD Ca was 1.8 g/kg for all. Hence, at the low Ca/P the increase in Ca was too low (or the increase in P too large) to fully utilise the increment in P. These results indicate that the calculated STTD Ca/P ratio of 1.13 (1.8/1.6) in the supplement was far from optimal whereas 2.25 (1.8/0.8) was more close to optimal. This result most likely reflects that when requirements for soft tissue gain are met, supplementary Ca and P are largely retained as hydroxyapatite in bone in a ratio of approximately 2.15.

A decrease in dietary Ca content at constant P content (Treatment 2 vs 1 and 6 vs 5) reduced the Ca concentration and enhanced the P concentration in the urine. Thus the reduction in Ca caused a relative surplus of digestible P that could not be retained in bone because of a deficiency in Ca. This effect was bigger in phytase-free diets than in phytase supplemented diets. The consequences for the optimal Ca/P are discussed below.

4.2.3 Towards an optimal ratio of calcium and phosphorous

The combined results of the realised dietary digestible Ca and P content (Table 11) and their urinary excretion (Appendix 6) form an important tool to optimise the Ca/P ratio in the diet. Overall, the urinary Ca content was high or very high for treatments with the high STTD Ca/P ratio of 1.6 to 1.8. On the other hand, the urinary P content was high or very high for treatments with the low STTD Ca/P ratio of 1.1 to 1.3. These results suggest that the optimum Ca/P was between 1.3 and 1.6 to minimise the excretion and make optimal use of both Ca and P.

The urinary Ca content was somewhat high in Treatment 1, with Ca and P at CVB-recommendations. Urine Ca content was further enhanced by phytase inclusion in the diet (Treatment 5). This was partly explained by the higher STTD Ca/P in this diet (1.68 vs. 1.59). The Ca excretion in the urine due to a high STTD Ca/P is not primarily a problem in the post-absorptive metabolism, but the high dietary Ca may hamper the digestibility of P and thus the STTD P supply as discussed above. The reduction in Ca content and Ca/P as in practical diets (Treatments 2 and 6) reduced the urinary Ca content in phytase-free and phytase-supplemented diets, respectively. The increase in urinary P content indicates that the reduced dietary Ca content limited the retention of P in body tissues and thus STTD Ca/P in these diets was below optimal. It is not quite clear why the urinary P content was higher in phytase-free than phytase-supplemented diets while the determined STTD Ca/P in these diets was similar (1.25 and 1.26, respectively). In contrast, the urinary Ca content was higher on phytase-supplemented diets. Overall, the higher dietary digestible Ca and P content in phytase-supplemented diets may have facilitated a higher retention in bone, but this would be expected to result in a lower Ca excretion. We can speculate that use of phytase via solubilisation of both Ca and P contributed to synchronisation in their absorption and post absorptive utilisation, while otherwise poorly soluble Ca would be absorbed more gradually than P and less optimally utilised. This hypothetical mechanism however requires further study. We cannot exclude that differences between the two locations, e.g. in growth performance (413 and 337 g/d in a four-week period) and bone and soft tissue retention played a role.

The high urinary P content and excretion in Treatments 2 and 6 can be reduced by a reduction in dietary P content as demonstrated by the low urinary P content in Treatments 3 and 7. However, this enhanced again the urinary Ca content as in Treatments 1 and 5. Moreover, the low urinary P content in Treatments 1 and 5 indicates that the pigs were able to (almost) completely retain the dietary P at the CVB recommended level in these treatments. Hence, a reduction in dietary P as in Treatments 3 and 7 would equally reduce P (and Ca) retention, most likely as mineral retention in bone. This will likely reduce bone strength. Although the minimum required bone strength is not well known, it would therefore be recommended to reduce the dietary Ca/P to a ratio between Treatments 1/5 and 2/6 that would be (just) adequate to optimally use dietary P without excessive Ca excretion in the urine. This approach is substantiated by the results of Treatments 4 and 8, with supplementary P added to the diets of Treatments 1 and 5. This resulted in an almost complete reduction of the high urinary Ca content in Treatments 1 and 5, indicating that the pigs were able to further enhance their Ca and P retention in the body above the level realised in Treatments 1 and 5. Thus Ca and P retention on Treatments 1 and 5 were (substantially) below maximum bone mineralisation. Based on this study, the optimal STTD Ca/P for bone mineralisation would be between 1.25 and 1.6. In a dose-response study with pigs from 11-25 kg González-Vega et al. (2016) observed an increase in P-retention and bone ash until a STTD Ca/P of approximately 1.35 in a diet with 3.6 g STTD P. In a study with 4 P levels and 5 Ca levels in a factorial arrangement Lagos et al. (2019) observed an increase in bone ash content with an increasing Ca content until a STTD Ca/P between 1.35 and 1.5 in diets with 3.3 or 4.2 g STTD P. Based on these results, we would recommend to use a STTD Ca/P of 1.4 as a minimum in (phytase-supplemented) diets for weaned pigs. A further reduction would likely result in an increase in P losses via the urine and a reduction in bone mineralisation.

4.3 Growth performance

4.3.1 Differences between locations

Overall the growth performance was higher in the experiment conducted by De Heus using phytase-free diets, as demonstrated by the higher ADG and ADFI and lower FCR. All experimental diets were produced in one feed production plant from the same basal diet and feed materials. Hence, diets only differed in supplemented phytase, Ca and P. Obviously, potential causes of differences in growth performance include all differences between the locations, e.g. housing, management, health status, age and body weight at weaning, genetic capacity of the pigs, etc. No conclusions can be drawn regarding the contribution of phytase to these differences.

4.3.2 Influence of calcium content and calcium to phosphorus ratio

The CVB recommendations are based on Ca and P retention in the body, including bone mineralisation. Requirements for maximum growth performance may be lower than for bone retention, as demonstrated for growing-finishing pigs by Bikker et al. (2013). The Ca recommendations for weaned pigs are considered relatively high for practical diets. It was suggested that a high dietary Ca content may hamper feed intake and gain of pigs, e.g. via the buffering capacity of the diet. Thus we hypothesised that a reduction in Ca content might improve growth performance. This was indeed to some extent confirmed in the present study. In the combined dataset, the reduction in Ca content enhanced the feed intake in the total experimental period from day 0-28 and the daily gain from day 0-14. In the period from day 14-28 no increase in ADG was observed while the FCR was somewhat increased by the reduction in Ca content. Although the overall analyses does not indicate a significant interaction between Ca and phytase inclusion, comparison of the two experiments indicated a more consistent increase in both ADFI and ADG with reduction of dietary Ca in the study by De Heus using phytase-free diets. This was partly caused by somewhat more pronounced effects in the five week period then the four week period at this location. In the study by ForFarmers using phytase supplemented diets, the overall effect of a Ca reduction on growth performance was small. These results suggest that the high total amount of Ca and P from limestone and MCP can have a negative impact mediated by a reduction in feed intake, in particular in phytase-free diets. Moreover, all gross dietary Ca levels were lower in phytase-supplemented diets (because of the contribution of phytase to the Ca supply) than in phytase-free diets. This may have contributed to the smaller beneficial effect of a (further) reduction of Ca in the phytase-supplemented diets. In conclusion, a reduction in dietary Ca content below CVB recommendations may improve growth performance in phytase-free diets, but the effect in phytase-supplemented diets may be minor.

The main effect of Ca as discussed here was determined at a constant Ca/P ratio, hence P was altered proportionately to Ca. In the present study a reduction in Ca/P, realised by an increase in dietary P increased the FCR, in particular in phytase supplemented diets. This indicates that in diets with relatively high Ca/P (based on CVB-recommendations) and hence relatively low P content, dietary P content did not limit animal performance, neither at the high (Treatments 1 and 5) nor at the low (Treatments 3 and 7) Ca content. Thus, we cannot confirm the hypothesis that a potential negative effect of a high dietary Ca content is mediated by a reduction in digestibility of P. In contrast to our study, a negative effect of a high dietary Ca content on gain and gain/feed was (partly) compensated by an increase in dietary P content in the study of Qian et al. (1996), González-Vega et al. (2016), Wu et al. (2018) and Lagos et al. (2019). The difference with our study was likely due to the selected dietary Ca and P contents. In Qian et al. (1996) and Lagos et al (2019) the negative effect of a high dietary Ca content on growth performance was prominent and much bigger than in our study at a digestible P content below 2.0 g/kg. The negative effect of Ca was much less when dietary digestible P was above 2.6 g/kg (Qian et al., 1996) or 3.3 g/kg (Lagos et al., 2019). Also in Wu et al. (2018) a substantial reduction in gain/feed was observed by a dietary Ca content of 9-12 g/kg when P was at NRC recommendations whereas only a small reduction in gain/feed was observed by an increase in Ca content to 12 g/kg when digestible P was 25-30% above recommendations. In their study the lowest digestible Ca/P ratio was approximately 1.7, which is close to the highest level in our study. González-Vega et al. (2016) observed a beneficial effect of extra P when STTD Ca/P was reduced from 2.2 to 1.8.

From these data we can conclude that the potential negative effect of a high Ca content on growth performance is mediated by the dietary P content and Ca/P ratio. The negative effect of high Ca is larger when dietary P is marginal or below requirements. In these conditions, the effect is mediated by a deficiency in P and can be largely compensated by an increase in dietary P content. In our study the effect of high Ca was small since the P supply was at or close to the requirements and the high ratio of digestible Ca/P of 1.6 was relatively low compared to studies that observed larger negative effects of Ca.

In practical diets, Ca content is often included at a level below CVB-recommendations without concomitant reduction in P content, thus reducing the Ca/P ratio. This was represented by comparison of treatment 2/6 (LCa, LCa/P) versus treatment 1/5 (HCa, HCa/P). The Ca reduction at constant dietary P content caused a higher feed intake and growth rate on phytase-free diets and a higher feed intake with similar growth rate in phytase-supplemented diets. The FCR was equal (De Heus) or slightly higher (ForFarmers) on the low Ca treatment. Thus again the benefit of the Ca reduction was somewhat larger in the phytase-free diets. This may be due to the lower actual STTD P content and the higher total Ca content compared to the phytase-supplemented diets. We further hypothesised that the use of these practical-type diets with low Ca content would result in a high P-excretion because of the low Ca/P and that a reduction of dietary P would reduce P-excretion without negative effect on growth performance. The latter was largely confirmed by the trial results. The reduction in STTD-P from 3.8 (Treatment 2/6, LCa, HCa/P to 3.0 g/kg (Treatment 3/7, LCa, LCa/P) did not reduce ADG and slightly improved the FCR in particular in phytase-supplemented diets. Nonetheless, as discussed in the previous paragraph, this reduction in Ca and subsequently P content would reduce Ca and P retention and bone mineralisation. Overall, the results indicate that the Ca content and Ca/P have a relatively small impact on growth performance within the range used in the present study, especially in phytase-supplemented diets. The reduction in Ca content enhanced the ADFI in both diets and the ADG in phytase-free diets without effect in phytase-supplemented diets. The Ca/P ratio can be reduced below the ratio adopted in de CVB-recommendations without loss in feed intake and gain, but with a minor increase in FCR.

In the studies cited above, a negative effect of dietary Ca on growth performance was observed in particular at (very) high Ca levels, i.e. above approximately STTD Ca/P of 1.8, in combination with a marginal P supply. González-Vega et al. (2016) determined the impact of dietary Ca content in diets with adequate, i.e. 3.6 g STTD P in pigs from 11 to 25 kg. In this study ADG and gain/feed (G/F) were unaffected by an increase in dietary Ca from 3.8 to 7.7 g/kg and reduced at 8.6 and 10.3 g/kg (i.e. above STTD Ca/P 1.50-1.55). In a study with weaned pigs from 11 to 22 kg, Lagos et al. (2019) determined the impact of STTD P and Ca in a 4 x 5 factorial arrangement. Growth performance increased quadratically with increasing Ca reflecting a beneficial effect at lower levels and a negative effect at higher levels. As in other studies, the negative effect of high Ca was enhanced at a low or marginal dietary P content. Based on our study and cited literature, we conclude that in diets with adequate P a negative effect of an increase in dietary Ca on growth performance is negligible below STTD Ca/P of 1.4 and small between 1.4 and 1.55. The latter is especially the case in phytase-supplemented diets because of the lower total (gross) Ca content.

4.4 Faecal consistency

Overall, the scoring for faecal consistency was somewhat higher (i.e. lower consistency) as registered by De Heus. Since this is a subjective scoring which is influenced by the persons conducting the observations, we cannot firmly conclude that this reflects an actual difference in faecal consistency between the locations. We expected that a high Ca content might reduce faecal consistency because of a potential influence on gut health, e.g. mediated by the buffering capacity. However, the dietary treatments only had minor effects on faecal consistency. In the study by De Heus a lower faecal consistency (higher faecal score) with high dietary Ca was only reported during the last observation on day 31. On location ForFarmers, a transient tendency for a lower faecal consistency (higher faecal score) with high Ca diets was observed on day 6, and a transient lower faecal consistency with a low Ca/P ratio (i.e. relatively high P content) on day 10. Nonetheless, the faecal dry matter content in week 4 was reduced on location ForFarmers with the high Ca, low Ca/P diet.

This diet had the highest Ca and P content. This suggests that the high mineral content may have increased the faecal water binding capacity, without obvious effect on faecal consistency. Overall, the results do not confirm that a reduction of dietary Ca content within the range in this study has a consistent beneficial effect on faecal consistency.

5 Conclusions and recommendations

5.1 Conclusions

- In this study using CVB recommendations as a reference, a reduction in total and STTD Ca in the diet enhanced feed intake in diets with and without microbial phytase but growth rate only in diets without phytase. Hence, dietary Ca content can be reduced without loss in performance.
- Negative effects of a high dietary Ca content most likely will be enhanced when diets are marginal or low in dietary P and when STTD Ca/P exceeds CVB recommendations.
- Results of our study and published literature in weaned pigs indicate that in diets with adequate P a negative effect of an increase in dietary Ca content on growth performance is negligible below STTD Ca/P of 1.4 and small between 1.4 and 1.55. This is especially the case in phytase-supplemented diets because of the lower total (gross) Ca content.
- In diets with reduced Ca and Ca/P ratio, dietary P is above optimal relative to Ca as indicated by an enhanced urinary P content. The results indicate that dietary P can be reduced in proportion to Ca without loss in performance.
- The reduction in Ca content and Ca/P enhanced urinary P content. Subsequent reduction in dietary P content resulted in a (large) reduction in P excretion, and in a drastic increase in Ca excretion. Both results indicate a reduction in Ca and P retention and bone mineralisation in the body. The results indicate that the optimum STTD Ca/P for retention is between 1.25 and 1.6. Including published studies in nursery pigs, the optimum is likely between 1.35 and 1.55.
- The reduction in dietary Ca content enhanced the ATTD of Ca in both phytase-free and phytase-supplemented diets. In phytase-free diets with reduced Ca content STTD of Ca was close to the adopted value of 60%, but in diets formulated at CVB recommendations STTD of Ca was substantially lower. This discrepancy between adopted and realised values was smaller in phytase-supplemented diets.
- The simultaneous reduction in Ca content and Ca/P ratio enhanced the ATTD of P in both phytase-free and phytase-supplemented diets.
- Faecal consistency was not substantially affected by the dietary Ca content and Ca/P ratio.
- A high dietary Ca content may reduce digestibility of organic matter, but this effect seems relatively small at practical inclusion levels.

5.2 Recommendations

- Results of our study and published literature in weaned pigs indicate that a high Ca to P ratio may reduce feed intake and body gain. This effect is larger in diets with marginal (low) P content. In diets with adequate P content, the negative effect of dietary Ca on growth performance is relatively small. Hence, adequate P supply according to recommendations is important.
- The minimum STTD-Ca/P for growth performance in weaned pigs is estimated as 1.25 to 1.4 (approximately 2.1 to 2.3 for total Ca/digestible P). A lower ratio is not recommended because of an increase in P losses via the urine and a reduction in bone mineralisation.
- In adequate P diets, the negative effect of dietary Ca on growth performance is negligible below STTD Ca/P of 1.4 and small between 1.4 and 1.55. A minimum STTD Ca/P of 1.4 (approximately 2.3 total Ca/STTD P) is recommended in diets for weaned pigs as a compromise between requirements for growth performance and for bone mineralisation and P utilisation.
- A ratio of STTD Ca/P of 1.55 (approximately 2.6 total Ca/STTD P) is recommended as a maximum for bone mineralisation, to minimise the negative effect of high Ca on growth performance and digestibility of P.
- We recommend the use of microbial phytase to assure adequate digestible Ca supply while minimising the total dietary Ca content.

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Appendix 1 Composition of diets

Table 1.1. *Ingredients and composition of nursery phase 1 (prestarter) and phase 2 (starter) basal diets without treatment specific supplementation of limestone, monocalcium phosphate and phytase*

Ingredient, %	**Phase 1**	**Phase 2**	**Nutrients, g/kg**	**Phase 1**	**Phase 2**
Barley	32.08	25.00	Crude ash	42	45
Wheat	25.00	29.23	Crude protein	174	181
Soybeans (heated)	9.57	10.00	Crude fat (hydr.)	83	80
Wheat middlings	6.00	6.00	Crude fibre	42	42
Whey powder conc. (CP 34%)	4.22	---	Starch (enz.)	359	358
Maize	4.15	5.00	Sugar	70	63
Soybean meal HP, (CP 49%)	1.00	4.22	NSP	163	163
Sunflower seed meal, (CP 37%)	2.50	3.50	EW	1.236	1.223
Choco biscuit mix	2.07	5.00	Na	2.6	2.4
Potato protein	2.00	2.21	Cl	4.0	4.1
Sugar beet pulp (<10% sugar)	1.50	1.00	dEB	166	166
Soybean oil	2.78	2.14	Ca	4.48	4.92
Coconut oil (raff.)	0.50	0.50	P	4.27	4.22
Fish oil	0.40	0.40	STTD-P	1.87	1.65
Sucrose	1.50	1.50	Lysine	13.4	13.4
Calcium formate	0.60	0.50	SID Lysine	12.2	12.1
Citric acid	0.50	0.50	SID LYS/EW	10.0	10.0
Lysine 50%, liquid	0.52	0.45	SID MET/LYS	0.39	0.39
L-lysine HCl	0.39	0.40	SID M+C/LYS	0.60	0.60
L-Threonine 98%	0.32	0.30	SID THR/LYS	0.68	0.67
DL-Methionine 98%	0.25	0.24	SID TRP/LYS	0.22	0.22
Valine 97%	0.20	0.17	SID ILE/LYS	0.46	0.48
Tryptophan 98%	0.09	0.09	SID LEU/LYS	0.84	0.88
Premix weaned pigs I	1.25	---	SID VAL/LYS	0.69	0.70
Premix weaned pigs II	---	0.35	SID HIS/LYS	0.26	0.29
Premix Vitamin E	0.05	0.05	C18:2	32	33
NaCl	0.37	0.37	Vitamin A	15000	12000
NaHCO3	---	0.20	Vitamin D3	1000	1000
Mono calcium phosphate	0.20	0.20	Vitamin E	150	150
Limestone, fine <.15 mm	---	0.48	Copper (CuSO4)	140	90
			Zink (ZnSO4)	137	92
Total	100	100			
Inclusion in experimental diets	96.36	97.19			

Table 1.2. *Ingredients (%) and nutrients (g/kg) of experimental phase 1 (prestarter) and phase 2 (starter) diets for weaned pigs.*

	De Heus, no phytase				ForFarmers, with phytase			
	T1	T2	T3	T4	T1	T2	T3	T4
Calcium	HCa	LCa	LCa	HCa	HCa	LCa	LCa	HCa
Ca/P	HCa/P	LCa/P	HCa/P	LCa/P	HCa/P	LCa/P	HCa/P	LCa/P
Phase 1 (prestarter)								
Basal mix II	96.36	96.36	96.36	96.36	96.36	96.36	96.36	96.36
Limestone	1.32	0.06	0.38	0.60	1.26	0.00	0.32	0.54
MCP	1.45	1.45	0.75	3.04	0.70	0.70	0.00	2.29
Phytase	---	---	---	---	0.16	0.16	0.16	0.16
Diamol	0.87	2.13	2.51	0.00	1.51	2.78	3.16	0.65
Total	100	100	100	100	100	100	100	100
Calculated nutrients								
P, g/kg	7.37	7.37	5.8	10.96	5.68	5.68	4.11	9.27
STTD-P, g/kg	4.5	4.5	3.2	7.5	4.5	4.5	3.2	7.5
Ca, g/kg	11.7	7.0	7.0	11.7	10.2	5.5	5.5	10.2
STTD-P from phytase, g/kg	-	-	-	-	1.4	1.4	1.4	1.4
Ca from phytase, g/kg	-	-	-	-	1.5	1.5	1.5	1.5
Phase 2 (starter)								
Basal mix II	97.19	97.19	97.19	97.19	97.19	97.19	97.19	97.19
Limestone	0.87	0.06	0.26	0.48	0.81	0.00	0.20	0.42
MCP	1.18	1.18	0.75	2.03	0.43	0.43	0.00	1.28
Phytase	---	---	---	---	0.16	0.16	0.16	0.16
Diamol	0.46	1.27	1.50	0.00	1.11	1.92	2.15	0.65
Titanium dioxide	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100	100	100	100
Calculated nutrients								
P, g/kg	6.75	6.75	5.79	8.67	5.07	5.07	4.10	6.98
STTD-P, g/kg	3.8	3.8	3.0	5.4	3.8	3.8	3.0	5.4
Ca, g/kg	10.0	7.0	7.0	10.0	8.5	5.5	5.5	8.5
STTD-P from phytase, g/kg	-	-	-	-	1.4	1.4	1.4	1.4
Ca from phytase, g/kg	-	-	-	-	1.5	1.5	1.5	1.5

Table 1.3. *Ingredients and composition of nursery phase 1 (prestarter) and phase 2 (starter) complete diets with treatment specific supplementation of limestone, monocalcium phosphate and phytase.*

Ingredient, %	**Phase 1**	**Phase 2**	**Nutrients, g/kg**	**Phase 1**	**Phase 2**
Barley	30.91	24.30	Crude ash	40	43
Wheat	24.09	28.41	Crude protein	167	176
Soybeans (heated)	9.22	9.72	Crude fat (hydr.)	80	78
Wheat middlings	5.78	5.83	Crude fibre	40	41
Whey powder conc. (CP 34%)	4.07	---	Starch (enz.)	346	348
Maize	4.00	4.86	Sugar	67	61
Soybean meal HP, (CP 49%)	0.96	4.10	NSP	157	158
Sunflower seed meal, (CP 37%)	2.41	3.40	EW	1.19	1.19
Choco biscuit mix	1.99	4.86	Na	2.5	2.3
Potato protein	1.93	2.15	Cl	3.9	4.0
Sugar beet pulp (<10% sugar)	1.45	0.97	dEB	160	161
Soybean oil	2.68	2.08	Ca	4.32	4.78
Coconut oil (raff.)	0.48	0.49	P	4.11	4.10
Fish oil	0.39	0.39	STTD-P	1.80	1.60
Sucrose	1.45	1.46	Lysine	12.9	13.0
Calcium formate	0.58	0.49	SID Lysine	11.8	11.8
Citric acid	0.48	0.49	SID LYS/EW	10.0	10.0
Lysine 50%, liquid	0.50	0.44	SID MET/LYS	0.39	0.39
L-lysine HCl	0.38	0.39	SID M+C/LYS	0.60	0.60
L-Threonine 98%	0.31	0.29	SID THR/LYS	0.68	0.67
DL-Methionine 98%	0.24	0.23	SID TRP/LYS	0.22	0.22
Valine 97%	0.19	0.17	SID ILE/LYS	0.46	0.48
Tryptophan 98%	0.09	0.08	SID LEU/LYS	0.84	0.88
Premix weaned pigs I	1.20	---	SID VAL/LYS	0.69	0.70
Premix weaned pigs II	---	0.34	SID HIS/LYS	0.26	0.29
Premix Vitamin E	0.05	0.05	C18:2	31	32
NaCl	0.36	0.36	Vitamin A	14454	11663
NaHCO3	---	0.19	Vitamin D3	964	972
Mono calcium phosphate	0.19	0.19	Vitamin E	145	146
Limestone, fine <.15 mm	---	0.47	Copper (CuSO4)	135	87
			Zink (ZnSO4)	132	89
Basal diet	96.36	97.19			
Sum of supplements as in Table 2	3.64	2.81			
Total	100	100			

1) From basal diet only, not including Ca and P from the supplements. For total Ca and (digestible) P, see table 2.

Appendix 2 Analysed composition of diets

Table 2.1. *Analysed nutrient content (g/kg) of experimental phase 1 (prestarter) and phase 2 (starter) diets for weaned pigs*

	De Heus, no phytase				ForFarmers, with phytase			
	T1	T2	T3	T4	T1	T2	T3	T4
Phase 1 (prestarter)								
Calculated								
Phosphorus	7.37	7.37	5.8	10.96	5.68	5.68	4.11	9.27
STTD-P	4.5	4.5	3.2	7.5	4.5	4.5	3.2	7.5
Calcium	11.7	7.0	7.0	11.7	10.2	5.5	5.5	10.2
bCalcium phytase	-	-	-	-	1.5	1.5	1.5	1.5
Analysed ForFarmers								
Dry matter	897	898	897	900	897	903	898	896
Ash	73	73	75	71	74	74	73	71
Calcium	11.9	7.1	7.7	11.4	10.9	6.6	6.3	10.4
Phosphorus	7.1	7.3	5.9	10.6	5.8	6.0	4.2	9.2
Phytase, FTU/kg	139	32	0	22	2176	2364	2935	1414
Analysed WLR								
Dry matter	907	911	910	912	913	918	916	910
Ash	89	89	90	87	87	89	93	88
Calcium	11.9	7.5	7.8	10.8	10.8	6.5	6.7	10.4
Phosphorus	7.39	7.57	5.93	10.29	5.74	5.79	4.07	9.02
Phase 2 (starter)								
Calculated								
Phosphorus	6.75	6.75	5.79	8.67	5.07	5.07	4.10	6.98
STTD-P	3.8	3.8	3.0	5.4	3.8	3.8	3.0	5.4
Calcium	10.0	7.0	7.0	10.0	8.5	5.5	5.5	8.5
bCalcium phytase	-	-	-	-	1.5	1.5	1.5	1.5
Analysed ForFarmers								
Dry matter	899	903	899	903	897	898	898	897
Ash	67	67	67	66	69	69	69	66
Calcium	10.0	7.1	7.1	9.7	9.2	6.2	6.4	8.9
Phosphorus	6.7	6.8	6.0	8.5	5.2	5.1	4.2	6.8
Titanium	1.77	1.84	1.78	1.74	1.78	1.84	1.84	1.76
Phytase, FTU/kg	22	50	56	40	3163	2356	3106	2052
Analysed WLR								
Dry matter	914	920	918	922	919	914	913	911
Ash	81	85	84	81	84	84	86	84
Calcium	10.2	7.3	7.4	10.1	9.3	6.3	6.6	9.1
Phosphorus	6.98	6.77	5.72	8.77	5.31	5.03	4.31	7.08
Titanium	1.9	2.1	2.0	1.8	1.9	2.0	2.0	1.9

Table 2.2. *Calculated and analysed content (g/kg) of proximate components in the experimental phase 1 (prestarter) and phase 2 (starter) diets for weaned pigs.*

	De Heus, no phytase				ForFarmers, with phytase			
	T1	T2	T3	T4	T1	T2	T3	T4
Phase 1 (prestarter)								
Calculated								
Crude ash	73	73	73	73	73	73	73	73
Crude protein	167	167	167	167	167	167	167	167
Crude fat	80	80	80	80	80	80	80	80
Crude fibre	40	40	40	40	40	40	40	40
Starch	346	346	346	346	346	346	346	346
Sugar	67	67	67	67	67	67	67	67
Analysed								
Dry matter	897	898	897	900	897	903	898	896
Ash	73	73	75	71	74	74	73	71
Crude protein (NIR)	161	162	161	164	161	165	164	161
Crude fat (NIR)	77	76	76	78	78	80	79	76
Crude fibre (NIR)	42	46	47	40	45	43	45	45
Starch (NIR)	350	327	330	338	339	341	331	331
Sugar (NIR)	48	50	51	46	49	52	51	47
Phase 2 (starter)								
Calculated								
Crude ash	68	68	68	68	68	68	68	68
Crude protein	176	176	176	176	176	176	176	176
Crude fat	78	78	78	78	78	78	78	78
Crude fibre	41	41	41	41	41	41	41	41
Starch	348	348	348	348	348	348	348	348
Sugar	61	61	61	61	61	61	61	61
Analysed								
Dry matter	899	903	899	903	897	898	898	897
Ash	67	67	67	66	69	69	69	66
Crude protein (NIR)	172	173	175	172	172	175	174	171
Crude fat (NIR)	76	78	77	77	77	78	79	77
Crude fibre (NIR)	43	41	44	44	44	42	40	43
Starch (NIR)	338	339	325	337	329	328	337	334
Sugar (NIR)	51	53	53	51	53	54	52	50

Appendix 3 Growth performance

Table 3.1. *Influence of dietary phytase (i.e. location), calcium content and calcium to phosphorus ratio on growth performance in piglets from day 0-14 post weaning.*

Phytase	Ca	Ca/P	TR	BW d14	SD BW d14	ADFI, g/d	ADG, g/d	FCR
No	High	High	1	12.6	1.26	362	276	1.31
		Low	4	12.4	1.05	367	270	1.36
	Low	High	3	12.7	1.19	377	283	1.33
		Low	2	12.7	1.12	374	283	1.33
Yes	High	High	5	10.0	1.21	240	175	1.38
		Low	8	10.0	1.35	255	172	1.49
	Low	High	7	10.3	1.19	259	187	1.40
		Low	6	10.4	1.43	263	179	1.47
SEM				0.19	0.088	8.0	6.4	0.027
	High	High		11.3	1.24	365	226	1.35
		Low		11.2	1.20	375	221	1.43
	Low	High		11.5	1.19	247	235	1.37
		Low		11.5	1.27	261	231	1.40
SEM				0.13	0.062	5.6	4.5	0.019
No		High		12.7	1.23a	370	280	1.32a
		Low		12.6	1.09a	370	277	1.34a
Yes		High		10.2	1.20y	249	181	1.39y
		Low		10.2	1.39z	259	176	1.48z
SEM				0.13	0.062	5.6	4.5	0.019
No	High			12.5	1.16	365	273	1.35
	Low			12.7	1.15	375	283	1.43
Yes	High			10.0	1.28	247	173	1.37
	Low			10.3	1.31	261	183	1.40
SEM				0.13	0.062	5.6	4.5	0.019
		High		11.4	1.21	310	230	1.36
		Low		11.4	1.24	315	226	1.41
SEM				0.09	0.044	4.0	3.2	0.014
	High			11.3	1.22	306	223	1.39
	Low			11.5	1.23	318	233	1.38
SEM				0.09	0.044	4.0	3.2	0.014
No				12.6	1.16	370	278	1.33
Yes				10.2	1.29	254	178	1.43
SEM				--	--	--	--	--
P-value								
Calcium				0.049	0.833	0.036	0.033	0.778
Ca/P ratio				0.824	0.717	0.381	0.343	0.003
Phytase x Ca				0.717	0.788	0.792	0.975	0.825
Phytase x Ca/P				0.581	0.011	0.445	0.822	0.058
Ca x Ca/P				0.665	0.337	0.399	0.958	0.179
Phytase x Ca x Ca/P				0.709	0.889	0.903	0.514	0.857

Table 3.2. *Influence of dietary phytase (i.e. location), calcium content and calcium to phosphorus ratio on growth performance in piglets from day 14-28 post weaning.*

Phytase	Ca	Ca/P	TR	BW d28	ADFI, g/d	ADG, g/d	FCR
No	High	High	1	20.3	756	546	1.39
		Low	4	20.0	771	545	1.42
	Low	High	3	20.3	778	543	1.43
		Low	2	20.6	798	561	1.43
Yes	High	High	5	16.9	660	492	1.35
		Low	8	16.9	682	493	1.39
	Low	High	7	17.4	700	508	1.38
		Low	6	17.2	688	487	1.42
SEM				0.28	13.8	11.8	0.020
	High	High		18.6	708	519	1.37
		Low		18.5	726	519	1.40
	Low	High		18.9	739	525	1.41
		Low		18.9	743	524	1.42
SEM				0.20	9.8	8.3	0.014
No		High		20.3	767	544	1.41
		Low		20.3	784	553	1.42
Yes		High		17.2	680	500	1.37
		Low		17.1	685	490	1.41
SEM				0.20	9.8	8.3	0.014
No	High			20.2	763	545	1.40
	Low			20.5	788	552	1.43
Yes	High			16.9	671	492	1.37
	Low			17.3	694	498	1.40
SEM				0.20	9.8	8.3	0.014
		High		18.7	723	522	1.39
		Low		18.7	735	521	1.41
SEM				0.14	6.9	5.9	0.010
	High			18.5	717	519	1.39
	Low			18.9	741	525	1.42
SEM				0.14	6.9	5.9	0.010
No				20.3	776	549	1.42
Yes				17.1	682	495	1.39
SEM				--	--	--	--
P-value							
Calcium				0.086	0.016	0.487	0.056
Ca/P ratio				0.846	0.250	0.936	0.082
Phytase x Ca				0.842	0.958	0.945	0.936
Phytase x Ca/P				0.764	0.541	0.261	0.280
Ca x Ca/P				0.827	0.460	0.908	0.503
Phytase x Ca x Ca/P				0.329	0.329	0.217	0.533

Table 3.3. *Influence of dietary phytase (i.e. location), calcium content and calcium to phosphorus ratio on growth performance in piglets from day 0-28 post weaning.*

Phytase	Ca	Ca/P	TR	BW d28	ADFI, g/d	ADG, g/d	FCR
No	High	High	1	20.3	559	411	1.36
		Low	4	20.0	569	408	1.40
	Low	High	3	20.3	577	413	1.40
		Low	2	20.6	586	422	1.39
Yes	High	High	5	16.9	450	333	1.36
		Low	8	16.9	468	333	1.42
	Low	High	7	17.4	479	348	1.38
		Low	6	17.2	476	333	1.43
SEM				0.28	9.6	7.5	0.017
	High	High		18.6	504	372	1.36
		Low		18.5	519	370	1.41
	Low	High		18.9	528	380	1.39
		Low		18.9	531	377	1.41
SEM				0.20	6.8	5.3	0.012
No		High		20.3	568	412	1.38a
		Low		20.3	577	415	1.39a
Yes		High		17.2	465	340	1.37y
		Low		17.1	472	333	1.42z
SEM				0.20	6.8	5.3	0.012
No	High			20.2	564	409	1.38
	Low			20.5	582	417	1.39
Yes	High			16.9	459	333	1.39
	Low			17.3	478	340	1.41
SEM				0.20	6.8	5.3	0.012
		High		18.7	516	376	1.37
		Low		18.7	525	374	1.41
SEM				0.14	4.8	3.8	0.008
	High			18.5	512	371	1.38
	Low			18.9	530	379	1.40
SEM				0.14	4.8	3.8	0.008
No				20.3	573	413	1.39
Yes				17.1	468	337	1.40
SEM				--	--	--	--
P-value							
Calcium				0.086	0.009	0.147	0.155
Ca/P ratio				0.846	0.232	0.642	0.005
Phytase x Ca				0.842	0.943	0.946	0.722
Phytase x Ca/P				0.764	0.902	0.329	0.082
Ca x Ca/P				0.827	0.377	0.946	0.229
Phytase x Ca x Ca/P				0.329	0.450	0.215	0.490

Appendix 4 Faecal consistency

Table 4.1. *Influence of dietary phytase (i.e. location), calcium content and calcium to phosphorus ratio on faecal consistency score in piglets from day 0-28 post weaning.*

Phytase	Ca	Ca/P	TR	Day 4	Day 6	Day 8	Day 10	DM, g/kg
No	High	High	1	1.25	1.06	0.88	0.88	259.8a
		Low	4	0.85	0.56	0.69	0.94	262.4a
	Low	High	3	1.31	0.94	0.94	1.00	261.8a
		Low	2	1.19	1.00	1.00	0.81	260.0a
Yes	High	High	5	1.06	1.31	0.25	0.69	263.2z
		Low	8	0.88	1.19	0.50	0.81	246.2y
	Low	High	7	0.94	0.88	0.44	0.38	262.4z
		Low	6	0.81	0.75	0.31	0.88	266.7z
SEM				0.246	0.237	0.169	0.144	3.90
	High	High		1.16	1.19	0.56	0.78	261.5
		Low		0.86	0.88	0.59	0.88	254.3
	Low	High		1.12	0.91	0.69	0.69	262.1
		Low		1.00	0.88	0.66	0.84	263.4
SEM				0.174	0.168	0.120	0.102	2.72
No		High		1.28	1.00	0.91	0.94	260.8
		Low		1.02	0.78	0.84	0.88	261.2
Yes		High		1.00	1.09	0.34	0.53	262.8
		Low		0.84	0.97	0.41	0.84	256.5
SEM				0.174	0.168	0.118	0.102	2.72
No	High			1.05	1.19	0.78	0.91	261.1
	Low			1.25	0.88	0.97	0.91	260.9
Yes	High			0.97	0.91	0.38	0.75	254.7
	Low			0.88	0.88	0.38	0.62	264.6
SEM				0.174	0.168	0.118	0.102	2.72
		High		1.14	1.05	0.62	0.73	261.8
		Low		0.93	0.88	0.62	0.86	258.8
SEM				0.123	0.118	0.084	0.072	1.92
	High			1.01	1.03	0.58	0.83	257.9
	Low			1.06	0.89	0.67	0.77	262.7
SEM				0.123	0.118	0.084	0.072	1.92
No				1.15	0.89	0.88	0.91	261.0
Yes				0.92	1.03	0.38	0.69	259.6
SEM				--	--	--	--	--
P-value								
Calcium				0.761	0.403	0.431	0.540	0.078
Ca/P ratio				0.232	0.307	1.000	0.221	0.276
Phytase x Ca				0.401	0.079	0.431	0.540	0.067
Phytase x Ca/P				0.761	0.780	0.599	0.068	0.221
Ca x Ca/P				0.629	0.403	0.792	0.759	0.123
Phytase x Ca x Ca/P				0.761	0.403	0.190	0.127	0.021

Appendix 5 Nutrient digestibility

Table 5.1. *Influence of dietary phytase (i.e. location), calcium content and calcium to phosphorus ratio on apparent total tract digestibility of organic matter (OM), calcium (Ca) and phosphorous (P).*

Phytase	**Ca**	**Ca/P**	**Treatment**	**OM**	**Ca**	**P**
No	High	High	1	79.93a	46.58	42.63
		Low	4	80.64ab	47.45	48.07
	Low	High	3	81.50b	55.47	39.32
		Low	2	81.43b	55.21	47.16
Yes	High	High	5	83.13yz	64.84	67.80
		Low	8	82.40y	62.77	71.18
	Low	High	7	83.08yz	75.62	70.55
		Low	6	83.58z	73.53	72.52
SEM				0.337	1.02	0.982
	High	High		81.53	55.71	55.22
		Low		81.52	55.11	59.62
	Low	High		82.29	65.55	54.94
		Low		82.50	64.37	59.84
SEM				0.238	0.722	0.695
No		High		80.72	51.02a	40.98a
		Low		81.03	51.33a	47.61b
Yes		High		83.11	70.23z	69.18y
		Low		82.99	68.15y	71.85z
SEM				0.238	0.722	0.695
No	High			80.28	47.01a	45.35b
	Low			81.47	55.34b	43.24a
Yes	High			82.77	63.80y	69.49y
	Low			83.33	74.58z	71.54z
SEM				0.238	0.722	0.695
		High		81.91	60.63	55.08
		Low		82.01	59.74	59.73
SEM				0.169	0.511	0.491
	High			81.52	55.41	57.42
	Low			82.40	64.96	57.39
SEM				0.169	0.511	0.491
No				80.87	51.18	44.30
Yes				83.05	69.19	77.51
SEM				--	--	
P-value						
Calcium				<0.001	<0.001	0.964
Ca/P ratio				0.680	0.223	<0.001
Phytase x Ca				0.197	0.094	0.004
Phytase x Ca/P				0.360	0.102	0.005
Ca x Ca/P				0.643	0.688	0.723
Phytase x Ca x Ca/P				0.038	0.701	0.175

Appendix 6 Urinary excretion

Table 6.1. *Influence of dietary phytase (i.e. location), calcium content and calcium to phosphorus ratio on urinary content (mg/kg) of Ca and P and Ca/P ratio.*

Phytase	Ca	Ca/P	Treatment	Ca	P	Ca/P ratio
No	High	High	1	554	231	8.8
		Low	4	163	2078	0.0
	Low	High	3	885	67	17.2
		Low	2	124	953	0.3
Yes	High	High	5	115	12	96.9
		Low	8	267	1066	0.3
	Low	High	7	1393	12	110.5
		Low	6	302	283	4.1
SEM				126	90/252	4.7
	High	High		854[b]	121[a]	53.0
		Low		213[a]	1572[c]	0.1
	Low	High		1139[c]	39[a]	63.9
		Low		214[a]	618[b]	2.3
SEM				89	64/178	3.3
No		High		721[b]	149[a]	13.0[b]
		Low		143[a]	1516[b]	0.1[a]
Yes		High		1272[z]	12[y]	103.7[z]
		Low		284[y]	675[z]	2.2[y]
SEM				89	64	3.3
No	High			358	1149[b]	4.4
	Low			506	507[a]	8.8
Yes	High			709	539[z]	48.6
	Low			847	148[y]	57.3
SEM				89	63	3.3
		High		997	80	58.4
		Low		214	1095	1.1
SEM				63	45	2.3
	High			534	844	26.5
	Low			677	327	33.1
SEM				63	45	2.3
No				432	828	6.6
Yes				778	343	53.0
SEM				---	---	---
P-value						
Calcium				0.110	<0.001	0.050
Ca/P ratio				<0.001	<0.001	<0.001
Phytase x Ca				0.954	0.050	0.516
Phytase x Ca/P				0.023	<0.001	<0.001
Ca x Ca/P				0.114	<0.001	0.191
Phytase x Ca x Ca/P				0.667	0.473	0.868

To explore the potential of nature to improve the quality of life

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